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SECTION MEETINGS

DISTRICT OF COLUMBIA	
U. S. Public Health Auditorium	December 5, 1940
SOUTHERN	
Louisiana State University	December 20, 1940
SOUTHERN CALIFORNIA	
Occidental College, Los Angeles	December 11, 1940

11869 P

A Dosage Response Equation for Androgen Assay by the Chick Comb Method.*

Franklin Hollander, Emanuel Klempner and
Robert T. Frank.

From the Laboratories of the Mount Sinai Hospital, New York.

In previous reports on the relation between dosage of crystalline androsterone (A)† and mean comb weight (*W*) of white leghorn chicks, we described experiments involving 582 chicks in 5 monthly series. Each series consisted of 6 "gamma-groups" of chicks;[1,2] *i.e.*, each bird received a total dose of 0, 10, 20, 30, 40 or 50γ androsterone in 0.35 cc of sesame oil, 0.05 cc being applied to the comb daily from the 3rd to the 9th day after hatching. The comb was excised and weighed on the 10th day.

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* This investigation was supported in part by a grant from the Friedsam Foundation. We also desire to express our thanks to Dr. Erwin Schwenk of the Schering Corporation of New Jersey for supplying us with the androsterone used in this investigation.

† The symbols used in this report are as follows:

A = androgen activity of the portion of specimen applied to *each* chick, expressed as micrograms (γ) of crystalline androsterone.
N = number of chicks treated; sex indicated by subscripts m and f.
W = comb weight.
W^2 = comb weight squared.
B_0 = initial body weight (3rd day).
B = terminal body weight (10th day).
S = standard error of estimate (a statistical measure of reliability).

A symbol in italics (*e.g.*, *W*) represents the mean value of this variable for a group of chicks treated similarly.

In the work now reported the number of series was increased to 10 (1439 chicks) over a period of 12 months, but in addition to W, A, N_m, and N_f, we recorded B_o and B. We also continued to run a control (zero γ) group of chicks with each series, as a measure of

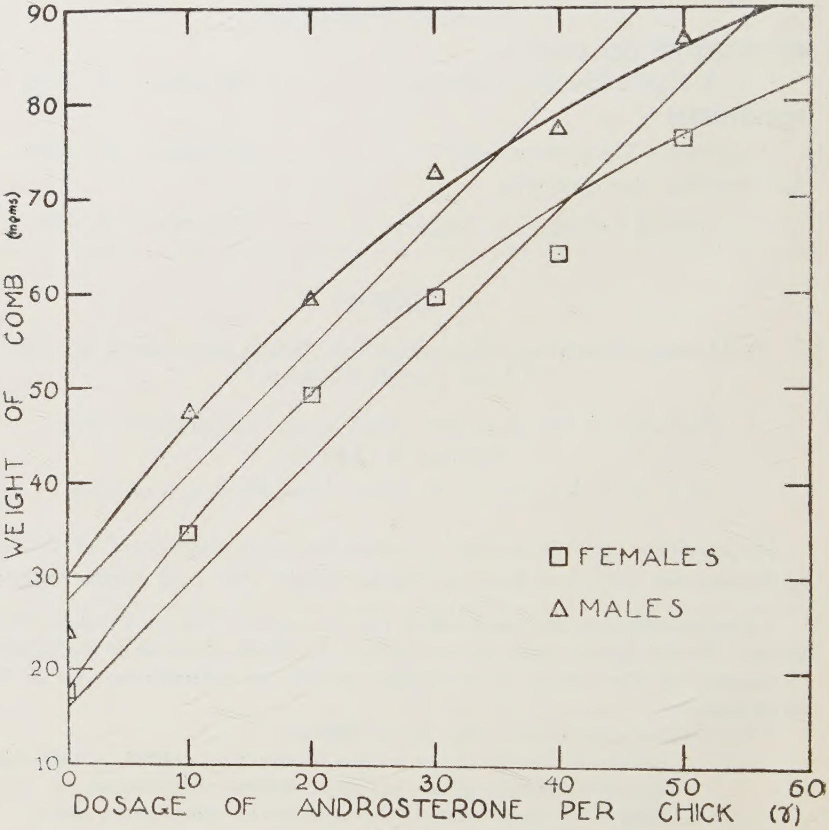

FIG. 1.

Relation between mean comb weight and dosage of androsterone. Combined data for 698 male and 741 female chicks (10 series).

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[1] Frank, R. T., and Klempner, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 763.

[2] Frank, R. T., Klempner, E., and Hollander, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 853.

variations in spontaneous comb growth activity associated with season and batch of chicks. Using these combined data, we investigated graphically and statistically the desirability of applying to W for any series and sex a simple correction for mean comb weight of the corresponding controls (W_c). Apart from sex difference, the improvement in the dose-response relation effected by this correction ($W - W_c$) was very small and probably negligible in the presence of corrections for B and B_o. Analysis of the data was therefore continued without such corrections. Graphs of W for each gamma-group (sexes separate) against A (Fig. 1) show that the data conform roughly to parallel straight lines, but a curvilinear fit may be closer. However, the graph for W against log A (Fig. 2) fits no better than the linear relation; this is confirmed by the identity of the corresponding correlation coefficients. Hence, adjustment for curvature must be made by the use of terms of the second order (see below).

Since the deviations of individual points in Fig. 1 from their respective lines result only in part from random variations in comb growth, we next tried to reduce these deviations by correcting for specific, uncontrollable variables. Employing the various zero-order correlation coefficients as criteria, we found that considerable improvement in reliability of the assay (*i.e.,* in S) can be effected by the use of individual chick data (both sexes combined), corrections for B_o and B, N_m and N_f, as well as W^2. Various functions of B_o and B and their squares afforded no significant improvement; likewise, a correction term for spontaneous comb growth (W_c) yielded such slight additional improvement in the presence of the other factors as to be negligible. Thus, instead of a simple linear relation between only 2 variables (W and A), we have evolved a single dosage-response equation which contains 7 variables without any bias in estimating their relative importance (*i.e.,* by multiple correlation):

$$A = 1.061W - 0.0043W^2 - 0.397B_0 - 0.267B + 14.75\frac{N_m}{N_m + N_f} + 18.54\frac{N_f}{N_m + N_f}$$

It is our practice to express W in milligrams, B_o and B in grams, and A in micrograms (γ).

Under our working conditions, such an assay possesses a coefficient of multiple correlation, $R_{\gamma.W,B,etc.}$, of ± 0.78 in the dosage range 0–50γ; S (for a single chick) is $\pm 10.9\gamma$. For 16 chicks this reliability measure becomes $\pm 2.7\gamma$, corresponding to a coefficient of variation (S expressed as percent of the mean γ-value) of $\pm 11.3\%$. Hence, of any *large* series of assays, practically none should differ

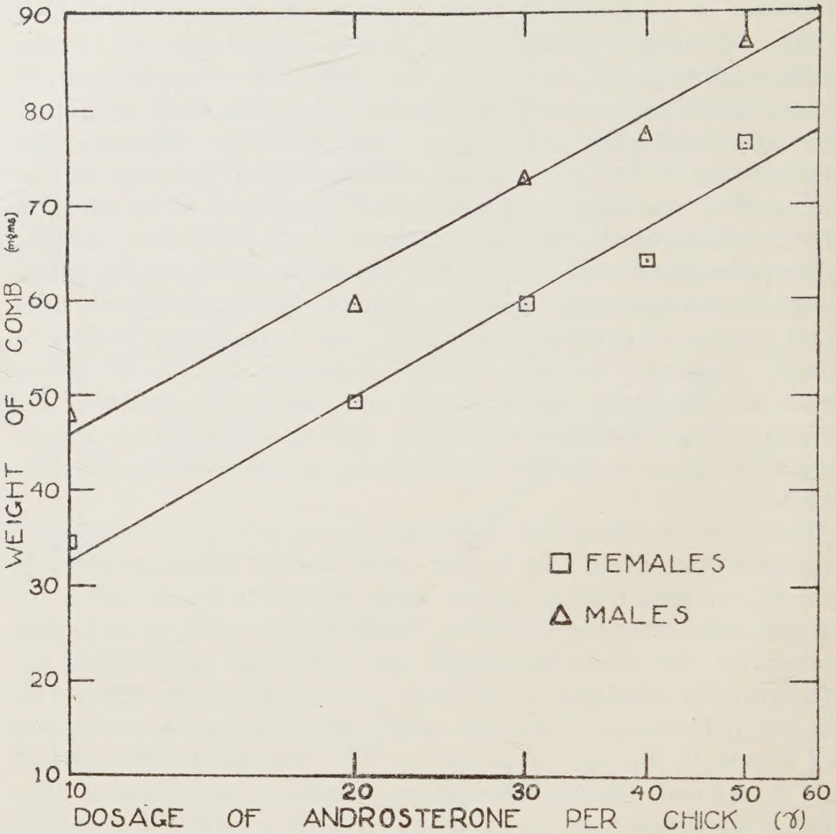

Fig. 2.

Relation between mean comb weight and log-dosage of androsterone. Combined data for 568 male and 575 female chicks (10 series).

from its "true" value by more than ±8.1γ (or 34%) *provided these assays and the experiments upon which the dosage-response equation was based constitute fair samples of the same total population.* In contrast with this, we may cite the reliability measures of our earlier procedure based on $(W - W_c)$, the mean comb weight for each gamma-group (in contradistinction to comb weights for individual chicks) adjusted only for a simultaneous control group. For all 60 gamma-groups involving 1439 chicks in 10 series (6 gamma-groups per series), the correlation coefficients were ±0.81 (males) and ±0.88 (females), and the corresponding S-values for a gamma-group containing 16 chicks were ±10.0γ and ±8.2γ. The root mean square of these S-values (9.1γ) is 3.4 times as great as the analogous value (2.7γ) found for assays based on our dosage-response equation, which demonstrates the improvement in reliability effected by adjustments with the several correction terms W^2, B, etc. Reducing the dosage range to 0-40γ decreases S to ±2.2γ, but since this corresponds to a coefficient of variation of 11.6% (compared with 11.3% for the longer range) it affords no actual improvement in reliability.

Concerning a unit of androgen activity for urine, we may define the *androsterone equivalent per liter* of any specimen as the number of tenths of a milligram (or hundreds of gammas) of crystalline androsterone which yield the same *adjusted* comb growth response as one liter of the specimen. Detailed statistical reports on this work are now in preparation.

11870

Leucocytosis Induced by Injection of Kaolin into Cisterna and Ventricles of Brain.

G. ROSENOW. (Introduced by B. Kramer.)

From the Department of Laboratories, The Jewish Hospital of Brooklyn, N. Y.

As has been shown in previous studies[1, 2] neutrophilic leucocytosis can be induced in rabbits by mechanical lesions in certain parts of the diencephalon, especially the hypothalamic region; the production of leucocytosis by bacterial proteins can be inhibited by the previous administration of narcotics such as luminal which incapacitates, functionally, the parts in question.[3] Thus, there seems to be no doubt that the diencephalon plays an important role in the regulation of the composition of the peripheral blood.

The nature of this regulation is not yet clear. It seems likely that the cerebrospinal fluid might be involved in the induction of cerebral leucocytosis.

Therefore, studies were now undertaken to determine whether a partial block in the flow of the cerebrospinal fluid would produce any characteristic changes in the number of leucocytes and the blood picture. I used the intracisternal or intraventricular injection of a suspension of kaolin in order to obtain stasis in the cerebrospinal fluid over a long period. This method was inaugurated for other

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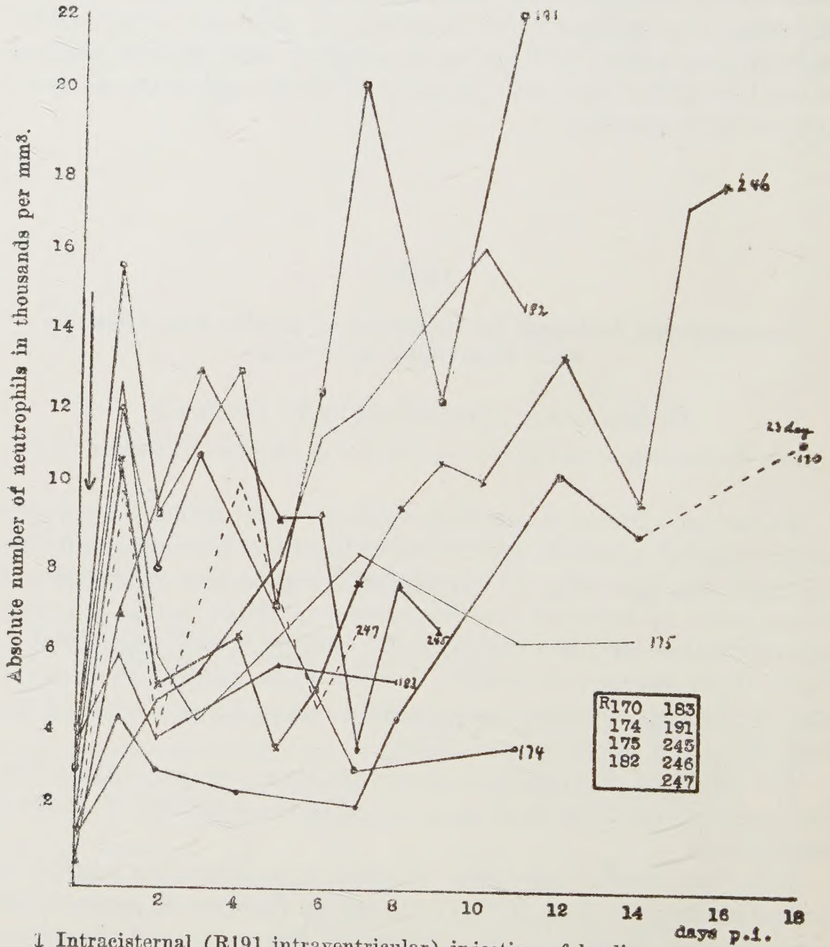
[1] Rosenow, G., *Z. f. d. ges. exp. Med.*, 1929, **64**, 452.

[2] Rosenow, G., *Z. f. d. ges. exp. Med.*, 1929, **65**, 557.

[3] Rosenow, G., *Verhandl. d. deutsch. Gesellsch. f. inn. Med.*, 1931, **43**, 75.

purposes by Dixon and Heller in dogs,[4] and by Griffiths, Jeffers and Lindauer[5, 6] in rats and cats.

Eighteen rabbits (2.5-4 kg) were used. After the normal level of the leucocytes (under the usual precautionary measures) was determined, 0.25-1.0 cc of a sterile 25 vol. % suspension of kaolin in saline was slowly injected intracisternally (in some experiments in



↓ Intracisternal (R191 intraventricular) injection of kaolin.

FIG. 1.

Absolute number of neutrophils, immature cells included, in thousands per mm³ in 9 rabbits.

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[4] Dixon, W., and Heller, H., *Arch. f. exp. Path. u. Pharm.*, 1934, **166**, 265.

[5] Griffiths, I. Q., Jr., Jeffers, W. A., and Lindauer, M. A., *Am. J. Physiol.*, 1935, **113**, 285.

[6] Lindauer, M. A., and Griffiths, I. Q., Jr., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 547.

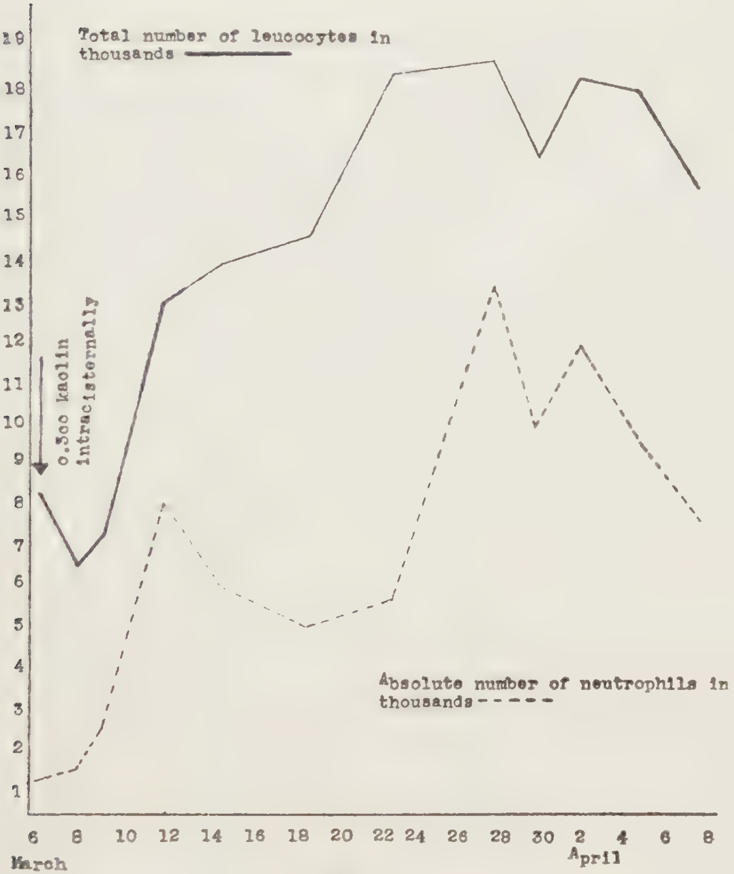

FIG. 2.

Rabbit No. 171 0.3 cc suspension of kaolin injected intracisternally.

one of the lateral ventricles). No narcotics were used. Blood was taken from the vein of the ear and leucocytes and differential counts (Wright and Giemsa) were made almost every day. Some of the animals died spontaneously, the others were killed after various periods of observation (6-30 days). Brain and medulla were examined macro- and microscopically after formalin fixation. In no case signs of infection or meningeal irritation were found. The results of 8 rabbits used are not included as the autopsy showed either coccidiosis or the rabbit did not survive for at least one week.

Results. In most of the experiments there appeared a marked neutrophilic leucocytosis on the 1st-3rd day after the kaolin injection. (Fig. 1). Usually this primary leucocytic response subsided after 2 to 5 days partly or completely, following which the number of neutrophilic leucocytes increased progressively during the next 10-20 days. Only 2 rabbits which survived more than 7 days had no leucocytosis; all the others showed a lasting and marked secondary leucocytosis which amounted to more than 100% of the initial value with a marked shift to the left. Fig. 2 gives an example of this type. In some experiments a few metamyelocytes and normoblasts were found in the peripheral smears at the peak of the leucocytosis.

The "primary" leucocytosis appeared sometimes very promptly after the injection. Table I shows the onset of the leucocytic response within 2 hr after the injection of kaolin into one of the lateral ventricles. The leucocytosis in this experiment was very marked. The number of segmented neutrophils increased from 3800 to 15500

TABLE I.

Rabbit No. 191. 11:25 injection of 0.65 cc of a 25% suspension of kaolin into the left lateral ventricle.

Date	Total Leuk.	Segm. neutrophils		Stab cells	
		%	Abs.	%	Abs.
5/9/40					
11:20 A.M.	9900	38.5	3812	1	99
11:25	Injection of 0.65 cc of kaolin into left ventricle.				
11:30	11450	38	4351	0.5	57
11:50	6300	38	2394	3	189
1:10	20950	74	15503	9	1885
2:10	15900	72	11448	7.5	1192
4:15	17600	75.5	13288	5.5	968
5/10	19050	75.5	14383	6	1143
5/11	17150	53.5	9175	2.5	429
5/13	19850	65	12903	1	198
5/14	12100	56.5	6837	3.5	423
5/15	18000	65	11700	4	720
5/16	27250	68.5	18666	5.5	1499
5/18	19550	58	11339	5	978
5/20	27750	67.5	18731	11	3053

Date	Lymphocytes		Monocytes		Basophiles		Plasma cells	
	%	Abs.	%	Abs.	%	Abs.	%	Abs.
11:20 A.M.	58.5	5792	1	99	0.5	49	0.5	49
11:30	55.5	6355	4	458	2	229	0	0
11:50	56.5	3560	0	0	2.0	126	0.5	31
1:10	15	3143	1.5	314	0.5	105	0	0
2:10	17	2703	1.5	239	1.5	238	0.5	80
4:15	17.5	3080	1	176	0.5	88	0	0
5/10	13	2476	2.5	476	3.0	572	0	0
5/11	35.5	6088	4	686	4.5	772	0	0
5/13	27.5	5459	2.5	496	4.0	794	0	0
5/14	32.5	3933	4.5	544	3.0	363	0	0
5/15	23.5	4230	4	720	3.5	630	0	0
5/16	18.5	5041	5	1363	2.5	681	0	0
5/18	30.5	5963	5	978	1.0	195	0.5	97
5/20	20	5550	0	0	1.5	416	0	0

in 1 mm^3 and the juvenile neutrophils (stabcells) from 99 to 1885 within 2 hr. Table I shows, furthermore, that there was first a short leucopenic stage as in most of the hitherto known forms of induced leucocytosis.[7]

In some instances (No. 170, 182) there was no primary leucocytic response after the injection of the kaolin and marked changes in the number of leucocytes and the blood picture did not appear before the 5th or 6th day.

Whether the mechanism of the primary and secondary leucocytosis is different remains questionable. Lindauer and Griffiths (*l.c.*) have found a pleocytosis of short duration in the cerebrospinal fluid in rats after injection of kaolin; however, it seems unlikely that the primary peripheral leucocytosis of the first days is due to an aseptic inflammatory irritation of the meninges as there were no signs of any meningeal irritation. For our problem the leucocytosis after the first days is more significant.

Summary. Intracisternal or intraventricular injection of a suspension of kaolin produces in rabbits a marked peripheral neutrophilic leucocytosis. These findings add another form of cerebrally induced leucocytosis to the other previously described; they furnish further support to the view of a central vegetative regulation of the cellular composition of the blood.

11871

Sulfanilamide, Sulfapyridine, Sulfathiazole and Sulfamethylthiazol in Experimental Gas Gangrene in Guinea Pigs.*

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(Introduced by E. W. A. Ochsner.)

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Since the publication of Domagk,[1] presenting the results of his experiments with Prontosil-S in streptococcal infections, numerous other investigators[2-6] have not only confirmed his work but also extended the scope of the usefulness of the derivatives of this drug.

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[7] Doan, C. A., Zerfas, L. G., Warren, S., and Ames, O., *J. Exp. Med.*, 1928, **47**, 403.

* Aided by a grant from the David Trautman Schwartz Research Fund.

[1] Domagk, G., *Deut. med. Wchnschr.*, 1935, **61**, 250.

The position which the drugs have in anærobic infections, especially that of gas gangrene, has not been clearly established. Bliss *et al.*,[5] and others[7, 8] described the effective action of sulfanilamide and its derivatives in experimental animals which had been injected with *Clostridium welchii.* Bohlman[9] reported 5 cases of gas gangrene treated successfully with sulfanilamide. Other authors[10, 11] have advocated the use of this drug in Clostridium infections. Unfortunately, the enthusiasm developing from these early reports was based largely on animal and *in vitro* experiments, which were not analogous to human infections. Recently, however, reports[12, 13] have appeared in the literature of the successful use of sulfanilamide in gas gangrene resulting from war injuries.

Clinical infections of the peritoneum due to the Clostridium group are rare, especially when consideration is taken of the frequency with which there is contamination due to the escape of intestinal contents following the traumatic or infectious break of the intestinal tract. Apparently, the peritoneum is able to resist contamination with *Clostridium welchii* more effectively than muscle tissue. The sacchrolytic nature of these organisms is well known and may partially account for this tissue susceptibility. Using sulfanilamide and sulfapyridine, Stephenson and Ross[14] were able to protect mice against several lethal doses of *Clostridium welchii* injected intraperitoneally. However, these same drugs proved ineffectual against a single lethal dose of the organisms intramuscularly.

A strain of *Clostridium welchii* which had been isolated from a fulminating case of gas gangrene obtained from Dr. Meleney's laboratory was used. Preliminary experiments showed this or-

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[2] Colebrook, L., and Kenny, M., *Lancet,* 1936, **1**, 1279.

[3] Long, P. H., Bliss, E. A., and Feinstone, W. H., *Pennsylvania Med. J.*, 1938-1939, **42**, 483.

[4] Long, P. H., and Bliss, E. A., *Canad. Med. Assn. J.*, 1937, **37**, 457.

[5] Bliss, E. A., Feinstone, W. H., Garrett, A. W., and Long, P. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 619.

[6] Lockwood, J. S., *Penn. Med. J.*, 1938-1939, **42**, 1444.

[7] Kendrick, Douglas B., *J. Clin. Invest.*, 1939, **18**, 593.

[8] Spray, R. S., *J. Lab. and Clin. Med.*, 1937-1938, **23**, 609.

[9] Bohlman, H. R., *J. A. M. A.*, 1937, **109**, 254.

[10] Kennedy, W. C., *Ill. Med. J.*, 1938, **73**, 260.

[11] Sadusk, J. F., Jr., and Manahan, C. P., *J. A. M. A.*, 1939, **113**, 14.

[12] Fuller, A. T., and James, G. V., *Lancet,* 1940, **1**, 487.

[13] Jocelyn Swan, R. H., *Brit. Med. J.*, 1940, **2**, 97.

[14] Stephenson, D., and Ross, H. E., *Brit. Med. J.*, 1940, **1**, 471.

ganism to be particularly virulent for guinea pigs. However, during the course of the experiments, this high degree of virulence diminished but was reestablished partially by serial animal passage.

This strain was found to be particularly virulent for guinea pigs when injected into the thigh muscles especially when followed by pressure, gently traumatizing the area injected. The organisms were grown in dextrose beef heart broth for varying lengths of time of 6 to 18 hr. In a few instances, the cultures were centrifugalized, the organisms separated, washed and resuspended in the proper dilutions of saline or supernatant broth. Solutions of sulfanilamide, sulfapyridine, sulfathiazole, and sulfamethylthiazol were injected intraperitoneally in 50 mg and 100 mg doses, the sodium salts of the last 3 drugs being used. Each animal received 3 doses of medication during the 24 hr period prior to the inoculation with organisms, and additional doses 3 times daily at 4 hr intervals during the day for varying lengths of time. By the injection of medication previous to inoculation with organisms, it was hoped to establish sufficient circulating medication before the development of an overwhelming

TABLE I.

Dose No. bacteria injected	Medication*	Dosage in mg	No. guinea pigs	No. surviving at hours								Survived	
				18	26	42	48	66	72	96	120	No.	Day
2200×10^6	San	50	16	8	7	4	2	0	—	—	—		
		100	6	5	4	0	—	—	—	—	—		
	Sp	50	16	10	8	2	2	0	—	—	—		
		100	6	3	2	0	—	—	—	—	—		
	St	50	16	16	13	4	3	2	2	0	—		
		100	6	6	4	0	—	—	—	—	—		
	Smt	50	16	14	11	5	4	1	1	1	0		
		100	6	5	1	0	—	—	—	—	—		
	Control		10	3	2	1	0	—	—	—	—		
			4	1	0	—	—	—	—	—	—		
1200×10^6	San	50	16	9	7	4	4	2	2	2	2	1	7
												0	8
	Sp	50	16	11	9	4	3	2	2	0	—		
	St	50	16	16	15	12	12	9	8	8	5	4	6
												2	7
												1	9
												0	15
	Smt	50	16	14	11	5	3	2	2	2	0		
	Control		10	5	2	0	—	—	—	—	—		
0	San		4	3 survived; 1 died on 5th day									
	Sp		4	”									
	St		4	”									
	Smt		4	2 ” 1 died on 3rd day and 2 on 5th day									

*Smt ≎ Sodium salt of sulfamethylthiazol.
St ≎ Sodium salt of sulfathiazole.
Sp ≎ Sodium salt of sulfapyridine.
San ≎ Sulfanilamide.

infection. Blood levels of the various drugs were not determined in any instance.

The guinea pigs inoculated with 0.4 cc of a dextrose beef heart broth culture died within 16 hours with a typical picture of gas gangrene. Because of the unusually high virulence, studies of progressively decreasing numbers of organisms obtained from 6 hour cultures, washed and resuspended in saline, showed that it was possible to kill guinea pigs with 0.12 cc of 320 × 10^6 organisms per cc, or a total of 40 million organisms. Using this smaller number, the survival time was increased to approximately 24 hours.

Experiment I. This experiment was performed in an attempt to determine the minimal number of *Clostridium welchii,* which when injected intramuscularly into guinea pigs receiving medication, would produce a fatal gas gangrene. It was found that the medications did not protect guinea pigs inoculated with 50 × 10^6 organisms, washed and resuspended in saline. Fewer organisms did not consistently produce death in either the medicated or control animals. None of those receiving sulfanilamide survived more than 48 hours. Sulfapyridine indicated an increased protective value in that it was able to protect all but one of the guinea pigs for 7 days or more; one, inoculated with 200 × 10^6 organisms, died within 42 hours. Of the 8 guinea pigs receiving sulfamethylthiazol, 3 died within 72 hours, 3 of the 5 remaining died on the 5th, 7th, and 8th days respectively, and 2 survived the infection. Sulfathiazole seemed to offer slightly more protection than the other three medications; of the 8 guinea pigs receiving this drug, 2 died on the 3rd day, one each on the 5th and 6th days respectively, and 4 survived the infection. Medication and organism controls were run throughout the experiment.

Experiment II. As the virulence of the strain had decreased, restoration was attempted by repeated animal passage. Since the virulence could not be brought back to its original level, it was necessary to increase the size of the inoculum to contain a minimum of 1200 × 10^6 organisms. Sixteen guinea pigs were used for each medication and each quantity of inoculum, with suitable controls. Of the 32 guinea pigs receiving sulfanilamide, 26 died within 48 hours, 4 within 66 hours, and the remaining two survived until the 7th and 8th days. Similar results were found in the sulfapyridine group, as all died within 4 days. Of the 16 animals receiving 2200 × 10^6 organisms and being treated with sulfathiazole, 13 died within 48 hours, one during the third day, and the remaining 2 died on the 4th day. Of the 16 guinea pigs receiving sulfathiazole and the smaller inoculum of 1200 × 10^6 organisms, 8 survived 3 days;

of these, 3 died on the 5th day, one on the 6th day, 2 on the 7th day, and 2 survived the infection but died of other causes on the 9th and 15th days. Of the group receiving sulfamethylthiazol, all 32 guinea pigs succumbed by the 5th day; however, the death rate was not quite as rapid as of those receiving sulfanilamide and sulfapyridine. Of the 20 organism controls, all but 4 died within 24 hours, and of the remaining 4, three died within 42 hours and one within 48 hours.

Due to the possibility that the dose of the medications employed in the preceding experiments was inadequate to demonstrate protection for the guinea pigs infected with *Clostridium welchii,* in another group of guinea pigs the dose of medication was increased to 100 mg, or twice that previously used. This was the only significant change in this experiment. However, even with this larger dose of medication, the results were similar to those observed in the experiments in which 50 mg of medication were used. All the animals died with gas gangrene within a period of 41 hours irrespective of medication. All organism controls were dead within 22 hours. Of the medication controls, the 2 receiving sulfamethylthiazol were dead on the 5th day, and the others were sacrificed on the same day for tissue study. From this experiment it can be concluded that the 100 mg doses of drug were no more effective than the 50 mg doses in protecting these guinea pigs.

In the experiments reported, autopsies were done on all of the animals which succumbed to the infection. Those receiving sulfanilamide and sulfapyridine developed a more extensive infection involving the musculature of the abdominal wall and spreading to the opposite side. The changes observed in the muscles appeared to be more profound; in many instances, the thigh muscles had undergone complete degeneration with lysis. In those pigs treated with sulfamethylthiazol and sulfathiazole, the lesion was different, in that the pathological changes were limited to the immediate adjacent muscle groups. Even though the lesion had spread to the abdomen in a few of these guinea pigs, in the majority of those receiving sulfathiazole and sulfamethylthiazol the involvement was not as extensive as those receiving sulfanilamide and sulfapyridine. *Clostridium welchii* was recovered from all lesions at autopsy.

From these experiments, it is impossible to conclude that these drugs are protective in *Clostridium welchii* infections of the thigh muscles of guinea pigs. The protection offered by these medications is very slight and varies somewhat with the medication used. Apparently, sulfanilamide and sulfapyridine intraperitoneally were of little benefit and in no way modified the course of the infection.

Even though most of the pigs treated with sulfathiazole and sulfamethylthiazol developed typical gas gangrene and succumbed, the development of the infection was delayed and in many instances was not as extensive as those seen in the guinea pigs treated with sulfanilamide and sulfapyridine. From this, the conclusion may be drawn that sulfamethylthiazol and sulfathiazole had slight therapeutic advantage over sulfanilamide and sulfapyridine in *Clostridium welchii* infections in guinea pigs.

11872 P

Further Experiments with Deplanted and Deranged Nerve Centers in Amphibians.*

PAUL WEISS

From the Department of Zoology, The University of Chicago.

A previous communication[1] has described the appearance of rhythmic spontaneous (=endogenous) activity, and later of reflexes, in fragments of spinal cord transplanted ("deplanted") to the dorsal fin of amphibian larvæ and provided with grafted limbs as effectors. A continuation of these studies has brought the following results.

A large section of *spinal cord* was deplanted to the fin and made to innervate 2 limbs, one grafted anteriorly and the other posteriorly. After re-innervation, prolonged seizures of spontaneous activity appeared in both limb grafts. During all major fits the beats of the 2 limbs occurred synchronously and with approximately proportional strength. This proves that in all actions, except very weak ones, the whole neurone pool of the grafted center discharges in unison. Reflexes likewise spread through the whole grafted center without localization.

Some limbs were deplanted along with their own spinal ganglia and cord segments, with all peripheral nerve connections being left intact. During the days following the operation this isolated reflex preparation gave orderly reflex responses. Gradually, however, the response deteriorated, assuming undifferentiated mass character with prolonged after-discharges, while at the same time spontaneous rhythmic seizures began to appear. This result demonstrates the direct relation between endogenous activity and central disorganization.

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* Investigations aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[1] Weiss, P., PROC. SOC. EXP. BIOL. AND MED., 1940, 44, 350.

In order to intensify the structural break-down normally occurring in the deplanted centers, fragments were mutilated in various ways prior to their transplantation (slashing; sucking through pipettes too small in diameter; mincing). Such grafts behaved in the same way as undisrupted ones: spontaneous seizures and reflexes appeared within a few weeks.

Spontaneous activity has also been obtained from deplanted fragments of the spinal cord of *adult* animals; it is not, therefore, restricted to the larval stage in which the previous observations were made.

In testing dorsal and ventral cord fragments separately, it was found that both can exhibit spontaneous seizures, although more experiments will be needed to determine whether their responses are exactly alike. At any rate, the fact that dorsal gray devoid of specialized motor cells does exhibit spontaneous activity, is in itself noteworthy. Similarly, reflexes were obtained from purely ventral fragments.

Deplanted fragments of *medulla oblongata* exhibit spontaneous activity of great intensity and usually very pronounced periodicity. However, none of the 8 cases thus far observed have shown reflexes of the kind regularly obtained in spinal cord grafts.

In confirmation of earlier observations, the composition of the blood was again found to affect the discharge threshold: invariably, exercise of the host body is followed by a marked intensification of spontaneous activity and reflex sensitivity in the deplants. Whether this effect is caused by anoxemia, CO_2, acidity, or metabolites, is for the future to decide.

In conclusion, these experiments amplify previously reported results in showing that deplanted pools of central neurones from a wide variety of sources display rhythmic spontaneous activity. We may assume that the factors generating this activity and determining its pulse are present in all gray matter, but are normally prevented from reaching discharge threshold by harnessing effects lying in the organization of the intact nervous system. The appearance of the rhythmic spontaneous discharges in the deplanted fragments may therefore be ascribed to the break-down (degradation) of the finer central organization. One factor affecting the discharge threshold has definitely been identified as humoral, inasmuch as the threshold fluctuates with the composition of the blood.

11873 P

Effect of Thyroidectomy upon Sexual Behavior of the Male Bovine.

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and W. L. Boyd.

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While many workers have reported on the effects of thyroidectomy upon fertility and sexual behavior for many species, no reference has been found to the effects of such operation upon the behavior of the bull. In general it is reported that thyroidectomy is followed by involution of the sex organs with reduced fertility and sterility.[1, 2, 3] Berliner and Warbritton[4] found that thyroidectomized male sheep continued to produce semen but the ejaculates were smaller and the sperm abnormalities increased.

This report deals with the observations over a period of 15 months following thyroidectomy of a male Jersey, the operation being performed at the age of 4 months, and the effects of administration of desiccated thyroid, dinitrophenol, benzidine sulfate and testosterone propionate.

For approximately 60 days following thyroidectomy the animal behaved normally. After that time, however, myxedemic symptoms developed rapidly. The skin became thick and puffy; the hair became dry, brittle and sparse; body fat increased and the general appearance of premature aging is presented. One of the most striking effects is that of reduced activity. Apathy, lethargy, increased susceptibility to cold and diminished reflexes are marked.

Blood analysis, made semi-monthly, has revealed a marked lowering in hemoglobin and amino nitrogen. Values for blood fat, cholesterol, glucose and non-protein nitrogen increased. Blood phosphorus, calcium, ascorbic acid, creatine, total nitrogen, globulin and albumin were normal.

While the gonads appeared to develop normally there was complete absence of libido at sexual maturity. His reaction in this particular was tested at frequent intervals with females in estrus and in no instance could he be induced to mate.

Periodic ejaculates were obtained by rectal manipulation of the

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[1] Krizenecky, J., *Verl.*, J. Springer, Berlin, 1932.

[2] Smelser, G. K., *Anat. Rec.*, 1934, **60**, 53.

[3] Zalasky, M., and Wells, L. J., *Anat. Rec.*, 1937, **69**, 79.

[4] Berliner, V., and Warbritton, V., *Proc. Am. Soc. Animal Prod.*, 1937, p. 137.

ampulla. Specimens so obtained were studied for normality and fertility. The amount of the ejaculates continued normal as did the number of spermatozoa. The sperm have also been normal in morphology, longevity and fertilizing ability. As a final test for normal sperm, semen samples obtained by manipulation were used in artificial insemination of cows and pregnancies have resulted.

After having determined the effects of thyroidectomy upon the sexual behavior it was decided to study the effects of administering desiccated thyroid, di-nitro phenol, benzedrine sulfate and testosterone propionate on the sexual behavior.

The test for sexual behavior consisted of observing the bull's reaction when present with females in estrus. Mounting of the female is considered as normal sexual behavior. Lack of interest in females in estrus and failure to mount them is considered as abnormal.

Oral administration of 25 g of desiccated thyroid over a period of 3 days restored normal activity and sexual behavior. These favorable effects were noticeable 24 hours after the thyroid feeding began and lasted for about 10 days after the last administration.

After a rest of 90 days from any medication and during which time he was watched for activity and frequently tested for sexual behavior, the effects of di-nitro phenol administration were tried. Administration of a sinlge 5-g dose of di-nitro phenol restored normal activity and sexual behavior in 12 hours. These effects of this drug disappeared completely in 36 hours after its administration.

Forty-five mg benzedrine sulfate were administered orally for each of 4 consecutive days. This drug had no noticeable effect upon either his activity or sexual behavior.

To test the effect of testosterone 450 mg of testosterone propionate pellets were implanted subcutaneously and a total of 300 mg of the hormone in sesame oil were injected intramuscularly over a period of 13 days. This treatment markedly increased the activity and restored normal sexual behavior which lasted for 3 days after cessation of the injections.

From this study it appears that thyroidectomy of the male bovine causes a complete inhibition of libido but has no effect upon spermogenesis or normality of the sperm. Desiccated thyroid and di-nitro phenol which increase metabolism restored normal activity and sexual behavior. Benzedrine sulfate was ineffective. Testosterone propionate improved both activity and sexual behavior.

We are indebted to the Schering Corporation for the testosterone propionate used in these experiments.

11874

Effect of Macromolecular Material from Chick Embryos on Growth Rate of Mouse Heart Fibroblast Cultures.*

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(Introduced by M. C. Winternitz.)

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Twenty-seven years ago Carrel and his coworkers[1] observed that extracts of embryonic tissues promote the proliferation of connective tissue cells *in vitro*. It was later found that extracts prepared from the organs of adult animals possess a similar activity. It has remained common practice to incorporate crude tissue extracts or organ emulsions into the blood plasma media employed for culturing tissues *in vitro*.[2]

Recently, Claude[3] isolated, by means of differential centrifugation at high speed, from normal 8-day-old chick embryos a macromolecular material possessing chemical and physical properties[3, 4, 5] similar to those of the purified Rous chicken sarcoma agent[6] and other materials obtained from normal and malignant tissues.[7] The purified chick embryo fraction was tested for possible tumor-producing activity in Plymouth Rock hens with negative results.[3]

The present experiments were undertaken to test the hypothesis that Claude's chick embryo fraction might be responsible for the growth-stimulating activity of embryo extracts.

Experimental. Preparation of Chick Embryo Fraction. The procedure employed in the present work was similar to that described by Claude,[3] but an air-driven ultracentrifuge[8] was substituted for the high-speed laboratory centrifuge attachment used by Claude.

Eight-day-old normal chick embryos (White Leghorn or Plymouth Rock) were freed from accessory material with aseptic precautions, ground to a paste with mortar and pestle and stored frozen at about —8° for from one to 3 days. The material was then weighed, finely

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* This investigation was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

[1] Carrel, A., *J. Exp. Med.*, 1913, **17**, 14; Ebeling, A. H., *ibid.*, 1913, **17**, 273.

[2] Parker, R. C., *Methods of Tissue Culture*, P. B. Hoeber, N. Y., 1938, p. 67.

[3] Claude, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 398.

[4] Claude, A., *Science*, 1939, **90**, 213; 1940, **91**, 77.

[5] Stern, K. G., and Duran-Reynals, F., *Science*, 1939, **89**, 609.

[6] Claude, A., *Science*, 1938, **87**, 467.

[7] Stern, K. G., and Kirschbaum, A., *Science*, 1939, **89**, 610.

[8] Beams, J. W., Linke, F. W., and Sommer, P., *Sci. Instr.*, 1938, **9**, 248.

ground with sterile sand and extracted with 6 times its volume of Tyrode[9] solution of pH 7.2. In order to eliminate tissue debris, the extract was centrifuged for 20 minutes at 2500 RPM in the horizontal head of a size 1 centrifuge (type S. B.) of the International Centrifuge Company. The opalescent supernatant fluid was then spun in sterilized lusteroid tubes in the concentration rotor of the air-driven high-speed centrifuge for 30 minutes at 30,000 RPM (67,000 G.). The resulting closely packed pellets, formed at the bottom of the tubes, were then resuspended in Tyrode solution. The suspension was freed from aggregated, coarse particles by a low-speed run in the laboratory centrifuge. The opalescent supernatant solution was withdrawn and subjected again to sedimentation in the ultracentrifuge. In all, the macromolecular material was purified by 3 or 4 cycles comprising a low- and a high-speed centrifugation. The final yellowish ultracentrifuge pellets, which had a translucent appearance and a gelatinous consistency were suspended in the same volume of Tyrode solution as the original embryo extract from which they were derived. In some experiments, samples of the solutions of the purified fraction were placed for 15 minutes in a boiling water bath, in order to determine the stability of the active principle. The solutions of the purified fraction were invariably opalescent and colorless.

Evaluation of Chick Embryo Fraction in Tissue Cultures. The methods of tissue culture employed in this laboratory have been presented in detail[10] The explants of the hearts of newborn mice in Carrel flasks were allowed to grow for approximately 72 hours in washed chicken plasma clots with a nutrient of rat serum diluted with 2 volumes of Tyrode. The colonies were then divided into control and experimental groups of at least 30 colonies each, and 0.3 cc of Tyrode, 0.3 cc of pellet, and 0.3 cc of pellet solution heated in a boiling water bath for 15 minutes were added to the respective flasks. The areas (A) of the explants were recorded and measured with a planimeter. The colonies were then allowed to grow for an interval of 30 hours and their areas (B) were again determined. The expansion rate factor (ERF) is defined as B — A/B × 100,000 divided by the number of hours in the experiment interval (30). The means of the ERF of the groups with their standard errors were compared for statistical significance by use of Fisher's[11] "t" factor.

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[9] Gey, G. O., and Gey, M. K., *Am. J. Cancer*, 1936, **27**, 45.

[10] Tennant, R., and Liebow, A. A., *Yale J. Biol. and Med.*, 1940, **13**, 39.

[11] Fisher, R. A., *Statistical Methods for Research Workers*, 5th Ed., Oliver & Boyd, London, 1934.

The results are recorded in Table I. In 9 experiments with material derived from 5 different batches of embryo extract the ERF of the pellet treated cultures was always greater than that of the Tyrode controls, in 5 instances with statistical significance.

The ERF of the cultures treated with the heated pellet materials even exceeded that of the unheated pellets, once in 5 instances with statistical significance (Exp. 296). The ERF of the former was in all 5 cases significantly greater than that of the controls.

Discussion. The association of a growth-stimulating principle present in embryo extracts with the macromolecular fraction isolated

TABLE I.
Effect of Macromolecular Material from Chick Embryo Extracts on Expansion Rate of Mouse Heart Fibroblast Cultures.

Source material and date	Exp. No.	1940	Tyrode control*	Pellet
274	274	1/16	963±27.5 (35)	1059±32.2 (35)
1/16				
287	300	5/3	1433	1477
2/28				
296	296	4/11	1482±20.8 (37)	1596±23.2 (38)
4/11	302	5/8	1727±24.5 (39)	1803±25.1 (38)
	308	6/1	1266±29.4 (42)	1390±28.2 (39)
313	313	6/29	1234±25.9 (38)	1312±29.5 (45)
6/29	316	7/8	1068±40.1 (39)	1381±30.6 (46)
315	317	7/29	1316±39.4 (35)	1316±28.1 (33)
7/12	318	9/8	1377±25.3 (33)	1451±20.6 (44)

Source material and date	Exp. No.	1940	Heated pellet	t	P
274	274	1/16		2.28 (ty, P)	.02
1/16					
287	300	5/3		(ty, P)	
2/28			1523	(ty, HP)	
296	296	4/11		3.67 (ty, P)	<.01
4/11			1695±24.6 (32)	2.91 (P, HP)	<.01
				2.15 (ty, P)	<.05
			1855±31.2 (36)	3.21 (ty, HP)	<.01
				3.04 (ty, P)	<.01
313	313	6/29		2.00 (ty, P)	
6/29			1392±32.3 (41)	3.82 (ty, HP)	<.01
	316	7/8		6.29 (ty, P)	<.01
			1393±38.4 (44)		
315	317	7/29			N.S.
7/12	318	9/8		2.27 (ty, P)	

*Expansion rate ractors are given with their standard errors. The number of colonies in the group is included between parentheses. t—Fisher's factor; P—probability of error; ty—Tyrode solution; P—pellet; HP—heated pellet; N.S.—not significant.

by Claude from such preparations by differential high-speed centrifuging which is indicated by the experiments here described opens the approach for an inquiry into its chemical nature. According to Claude[3, 4] this material, like similar fractions obtained from other tissues, consists essentially of a phospholipid portion associated with a nucleoprotein of the ribose type. However, this does not necessarily mean that the *intact* complex is required for the effect of this material on explanted cells. The observation that the heated pellet material exhibits even greater growth stimulation indicates that the heat-stable portion of the material (possibly the lipoid or the nucleic acid component) is sufficient to produce the effect. Moreover, the macromolecular nature of the intact complex makes it highly improbable that it could enter the cells by diffusion without preceding dissociation into smaller units.

Investigations concerning this increase in potency after heating are now in progress particularly in relation to possible liberation of nucleic acid[12] and of vitamin H.[13] The supernatant after the first ultracentrifugation is also under study since it has been found to be variable in its properties in the experiments conducted to date.

While this work was in progress, the attention of the writers was directed to a brief communication by A. Fischer[14] dealing with the purification of the active fraction present in beef embryo extracts. This worker finds that the activity appears to be associated with a nucleoprotein of the ribose type. The heat-lability and the destruction of the biological activity by tryptic digestion in his experience indicate that a protein forms an integral part of the active principle. Mention is also made of the fact that it is rather easily sedimented in the ultracentrifuge.

Summary. It is shown that a *macromolecular* fraction isolated from chick embryo extracts by differential high-speed centrifugation essentially according to Claude, exerts a distinct growth-stimulating effect on cultures of mouse heart fibroblasts.

The writers wish to acknowledge the technical assistance of Miss E. Carter and of Mr. H. Nelson in these experiments.

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[12] Claude, A., and Rothen, A., *J. Exp. Med.*, 1940, **71**, 619.

[13] György, P., Melville, D. B., Burk, D., and duVigneaud, V., *Science*, **1940**, **91**, 243.

[14] Fischer, A., *Nature*, 1939, **144**, 113.

11875

Poliomyelitis Virus Acclimated to Small Laboratory Animals.*

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Flexner's M.V. strain of poliomyelitis can produce paralysis in Eastern cotton rats.[1] Another experiment was started on 8/13/40 to see if our previous results could be duplicated.[1] The usual injections of cord brain suspension in these experiments were 0.06 cc intracerebrally (IC), 0.06 cc intranasally (IN) and 0.5 cc subcutaneously (SQ) of a 10% suspension in "enteric organism filtrate." The starting injection in the first generation was made with monkey cord. Each subsequent generation got the rat cord and brain of the preceding generation. Ten animals in Generation (Gen.) I were injected. All died between the 16th and 30th day; none had paralysis. Three animals in Gen. II were injected on 8/29 with Gen. I rat which died on the 16th day. These animals died between 42 and 46 days. Three animals in Gen. III were injected on 10/16 with a combination of all 3 animals from Gen. II. This time an additional injection of 1.0 cc was made IP. Two animals died on the 3rd and 1 on the 5th day; all were weak; none had paralysis. Three animals in Gen. IV were injected on 10/21 with a combination of all 3 rats of Gen. III. Twenty per cent cord suspensions were used in this experiment. One animal died on the 2nd, 1 on the 7th and 1 on the 8th day; the latter 2 were weak. Gen. V: On 10/30, 2 animals were injected with a 10% saline suspension combination from Gen. IV—the second 2 rats above. One animal was weak on the 5th and 1 had definite paralysis on the 6th day. Gen. VI.: The 2 animals injected on 11/7 with Gen. V had paralysis on the 7th day. Gen. VII: The 2 animals injected on 11/18 with Gen. VI had paralysis on the 7th day. Thus, this method of isolation was successful a second time.

An attempt was made to adapt Flexner's M.V. strain to cotton rats by a modified procedure. The idea was to condition the rats by injecting them with "enteric organism filtrate." *B. coli* isolated from one of the rats was used to make up the enteric organism filtrate.[1] On 8/13, 8/19, 8/24, 8/27, 8/31, 9/3 and 9/6, 3 rats

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* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

[1] Toomey, John A., and Takacs, William S., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 364.

were injected SQ with 0.5 cc filtrate. A second group of 5 animals was injected similarly on 8/31, 9/3, 9/6, 9/10, 9/13, 9/17 and 9/30. Monkey cord virus was injected in the usual way—0.06 cc IC, 0.06 cc IN, 0.5 cc SQ and 1.0 cc IP. Five rats died, 1 each on the 5th, 6th, 7th, 14th and 31st days following the cord injections. The other animals were weak. A 20% saline suspension from these Gen. I rats dying the 5th, 6th and 7th days was injected into 3 animals. Two animals remained well. One died. A 10% suspension of cord of the latter was injected into 5 other animals, without any "takes". The modified method did not help us to get successful "takes", and the experiment must be regarded as negative.

The acclimated rat cord virus was not lymphocytic choriomeningitis, for 1 cc of a 10% suspension of rat cord and brain of the 11th lineal generation transfer injected SQ into 20 guinea pigs failed to produce a disease condition in any of the animals. A neutralization test was done with lymphocytic choriomeningitis antiserum in mice, using a serum obtained from Dr. Thomas M. Rivers of the Rockefeller Institute capable of neutralizing 10,000 units or more of WE virus strain per cc. Twice the volume of the serum was combined with one volume of 30% ground mouse cord (1 cc of serum plus 0.5 cc 30% mouse brain virus); the whole was shaken in a mixing machine for 1 hour in the icebox and incubated for 1 hour at 37°C. The control injection suspension was made up with twice the volume of normal saline. The dose was 0.03 cc injected IC. A negative control experiment was done with 30 other animals wherein 0.03 cc of saline only was injected. There was a 10% accident rate following the latter injections. The results were clear-cut. Lymphocytic choriomeningitis antiserum instead of neutralizing seemed to boost the pathogenicity of the mouse cord suspensions, for by the 5th day 100% of the animals injected with this combination had died. One hundred per cent of the controls died by 11/26, 10 days after the experiment was begun. Thus, this acclimated virus was not a strain of lymphocytic choriomeningitis.

Could this acclimated virus be transferred from cotton rats to monkeys? There were 3 monkeys in Gen. I. On 8/16, 1 was injected IC with 1 cc of a 10% suspension of the cords and brains of cotton rats from the 6th lineal generation transfer. On 8/27, it developed quadriplegia. A second animal was injected on 9/9 with the same amount of virus in "filtrate" from the 7th lineal gen. It developed quadriplegia on 9/16. A third animal was injected IC with 1 cc of a 20% saline suspension of rat cord and brain from the 7th lineal gen. and developed quadriplegia on 9/17. Three monkeys were injected in Gen. II—the first on 9/12 with 1 cc of a 20% monkey cord suspension IC from Gen. I and 9/24 with the same amount IC, IP and SQ. On 9/30 and 10/3, it was again injected with 1 cc of a 10% suspension IP and SQ. On 10/22, it died from miliary tuberculosis. Some weakness present might have been due to poliomyelitis, but it was not definite. A second animal was injected on 9/24 with 1 cc of a 10% saline suspension of cord from Gen. I monkey. On 9/24 and 10/2, it received 1 cc of a 10% saline suspension IC. On 10/6, it developed paralysis, chiefly of the upper extremity and neck muscles. A third animal received 1 cc of a 10% suspension in "filtrate" from Gen. I on 9/21 and 10/1. It died from miliary tuberculosis on 10/3. Gen. III: On 10/6, a monkey was injected IC with 1 cc of a 10% saline monkey cord suspension of the second animal in Gen. II and reinjected IC, SQ and IP with the same amount on 10/16. On 10/23, it developed paralysis. Gen. IV: A monkey was injected IC and IP with 1 cc of a 20% saline suspension of the lumbar cord obtained from the animal that died in Gen. III and with 2 cc SQ on 10/23. It received 2 cc IP on 10/31 and 11/4. It developed quadriplegia on 11/7. Gen. V: On 11/11, another monkey was injected IC with 1 cc of a 20% cord suspension of the animal dying in Gen. IV. It developed quadriplegia on 11/22.

Although clinical poliomyelitis was produced, the animals, excepting the monkey in Gen. V, were usually injected several times with a brain cord antigen. Hurst[2] has shown that repeated injections of normal rabbit brain tissue might cause paralysis in the homologous animal. Rivers, Sprunt and Berry[3] repeatedly injected 8 monkeys with emulsions and alcoholic suspensions of rabbit brain, and in 2 animals paralysis was produced. The experimental animal does not develop paralysis after a primary injection. Although the animal in Gen. V developed paralysis after one injection and thus met possible objections because of nonspecific factors, another experiment was performed. On 11/9, a monkey was injected IC with 1 cc of a 10% suspension and IP with 2 cc of cotton rat brain and cord, not from the 6th but from the 11th lineal gen. On 11/16 it developed quadriplegia.

Thus, it has been shown (1) that the original experiments with Flexner's M.V. strain could be repeated in the cotton rat; (2) that rat cord virus obtained from paralyzed animals was not lymphocytic choriomeningitis; (3) that this acclimated rat cord virus could be transferred to monkeys.

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[2] Hurst, E. Weston, *J. Hyg.*, 1932, **32**, 33.

[3] Rivers, Thomas M., Sprunt, D. H., and Berry, G. P., *J. Exp. Med.*, 1933, **58**, 39.

In collateral experiments, we attempted to acclimate Flexner's Philadelphia virus strain to the cotton rat.

Gen. I: On 8/31, 5 cotton rats were injected with a 10% suspension of monkey cord (Philadelphia poliomyelitis strain) in "enteric organism filtrate". The original strain was obtained from Dr. Irving J. Wolman, The Children's Hospital, Philadelphia. The doses in this and in the following animals were: 0.06 cc IC, 0.06 cc IN and 0.5 cc SQ. Four of the 5 animals died—1 the 6th, 1 the 14th and 2 on the 29th day following. No animal was paralyzed. Gen. II: (a) The cords from the first 2 animals that died in Gen. I were ground in "filtrate" and injected in 3 other rats on 9/20. One animal died on the 29th day. (b) On 10/3, 3 other animals were injected with a combination of cords and brains of animals from Gen. I in filtrate. There was an added injection of 1.0 cc IP. Three rats died —1 in 24 hours and 2 within 5 days after the injections. Gen. III: A 10% saline suspension of the cords and brains of the animals in Gen. II that died on the 5th day was injected into 5 animals on 10/10. One animal was found dead on the 2nd, 1 the 5th and 1 the 3rd day; the latter had had paralysis of the front paw. Two died on the 4th day and had weakness. Gen. IV: A 20% saline suspension of the cords and brains of those rats of Gen. III that died on the 3rd, 4th and 5th days was injected into 4 animals on 10/18. The same percentage suspension used in this experiment was used in the next 4 generations. All the animals died—1 the 6th, 1 the 7th and 1 on the 9th day following the injection. All had weakness. Gen. V: On 10/23, 2 rats received material from the first animal that died on the 5th day in Gen. IV. Both died—1 the 5th and 1 on the 11th day. Both were weak. Gen. VI: On 10/28, 2 rats received material from the animal that day on the 5th day in Gen. V—1 died on the 3rd and the other on the 7th day with paralysis. Gen. VII: On 11/4, 2 rats received material from the animal of Gen. VI that died on the 7th day. They died on the 5th day with paralysis. Gen. VIII: In this experiment, it was thought best not to inject the animals IP. This added dose might have been successful in breaking down the general resistance, but when the virus has become acclimated, the additional IP injection is apt so to accelerate the disease that an animal would sometimes die before paralysis would be appreciated. On 11/19, 3 animals were injected with material from the 7th gen. All developed paralysis and died on the 5th, 6th and 7th days after injection.

Thus, Flexner's Philadelphia, as well as his M.V. strain, can be made to produce paralysis in the cotton rat.

11876

Acute Toxicity of Monacetin, Diacetin and Triacetin.

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Experimental studies of the toxic effects of "industrial solvents" are of considerable importance since some have been used as vehicles or solvents for medicinal substances in recent years. The glycols and their related compounds have been studied extensively.[1-5] On the other hand, the acetins have not received the attention they deserve. Diacetin or glyceryl diacetate has been used as a solvent for camphor[6] and more recently monacetin of glycol for vitamin K.[7] Latven and Molitor[8] have found the LD_{50} of diacetin to be 3.5 cc per kilo on subcutaneous injection in white mice. The narcotic effects[9] of monacetin and triacetin, as well as diacetin, were studied in 1899 by Meyer. Quantitative studies on their comparative toxicity are lacking, however. On account of ease of preparation and of their possible usefulness as substitutes for other organic solvents, a study of the acute toxic effects of these compounds was made in rats and mice.

Monacetin, diacetin and triacetin were prepared according to the methods of Schuette and Sah[10] and Schuette and Hale.[11] The purity of the compounds was established by comparison of their physical constants with those given in standard references.[12]

Inbred albino rats weighing usually from 200 to 350 g and inbred

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[1] Hanzlik, P. J., Seidenfeld, M. A., and Johnson, C. C., *J. Pharm. and Exp. Therap.*, 1931, **41**, 387.

[2] von Oettingen, W. F., and Jirouch, E. A., *J. Pharm. and Exp. Therap.*, 1931, **42**, 355.

[3] Haag, H. B., and Ambrose, A. M., *J. Pharm. and Exp. Therap.*, 1937, **59**, 93.

[4] Hanzlik, P. J., Newman, H. W., Van Winkle, W., Jr., Lehman, A. J., and Kennedy, N. K., *J. Pharm. and Exp. Therap.*, 1939, **67**, 101.

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[9] Meyer, H., *Arch. f. exp. Path. Pharmakol.*, 1899, **42**, 109.

[10] Schuette, H. A., and Sah, P. P. T., *J. Am. Chem. Soc.*, 1926, **48**, 3161.

[11] Schuette, H. A., and Hale, J. T., *J. Am. Chem. Soc.*, 1930, **52**, 1978.

[12] Prager, B., and Jacobson, P., *Beilsteins Handbuch der Organischen Chemie*, Berlin, Springer, 1922.

white mice* weighing usually from 17 to 21 g were used as test objects. At least 10 animals were used for each dose level. For injection into rats each acetin was mixed with equal volumes of distilled water to which 2% by volume of a 3 M phosphate buffer solution[13] was added to make the pH approximately 7 (bromthymol blue). In this medium diacetin and triacetin were not completely soluble. Care was taken to insure an even mixture before each injection and not more than 1 cc was given at one site. Mice were injected with acetins in 1:5 or 1:10 dilutions. These contained 1% by volume of the buffer solution. In all animals injections were given subcutaneously. Rats were observed for 15 days and mice for 5 days after a single injection.

Animals dying during the period of observation were examined and the following organs were removed for microscopic study: lungs, heart, liver, spleen and kidney. Gross observations of animals poisoned by monacetin revealed dilatation of the heart, diffuse congestion of the lungs, and in 2 rats, a small amount of fluid in the pleural cavity. In animals dying as a result of the toxic effects of diacetin and triacetin hemorrhagic areas in the lungs appeared frequently. Preliminary studies of sections of tissues showed some cloudy swelling of the convoluted tubules of the kidney and in some cases the lumen was filled with casts. Hydropic degeneration and necrosis of the tubules was noted in some areas. The liver appeared to be congested.†

The results of this study of the acute toxicity of the acetins in

TABLE I.
Acute Toxicity of Monacetin, Diacetin and Triacetin in Inbred Rats and Mice.

	Rats			Mice		
	Dose, cc/kg	No. of animals	No. died	Dose, cc/kg	No. of animals	No. died
Monacetin	3.0	10	0	2.0	10	2
	5-6	10	5	4.0	10	6
	7.5-10	10	10	6.0	10	10
Diacetin	2-3	15	0	2.0	10	0
	3.5	10	4	2.5	10	6
	4.0	10	5	3-4	10	9
	5-10	10	10			
Triacetin	2.0	10	1	1.0	10	2
	3.0	10	6	2.0	10	2
	4-10	10	10	2.5	10	7
				3.0	10	10

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* This strain of inbred mice was kindly supplied by Dr. A. B. D. Fortuyn.

[13] Butler, A. M., and Montgomery, H., *J. Biol. Chem.*, 1932, **99**, 173.

† We are indebted to Dr. C. H. Hu who confirmed these findings.

rats and mice are summarized in Table 1. The order of toxicity of the acetins appears to increase with the degree of acetylation and is approximately in the ratio of 1.0:1.4:2.0 in rats and of 1.0:1.4:1.5 in mice. Comparative studies of the subcutaneous toxicity of ethylene glycol indicate that monacetin is less toxic for rats and mice. Diacetin would appear to be approximately as toxic and triacetin apparently is more toxic than ethylene glycol.

Animals receiving fatal doses usually died in from 20 minutes to 3 or 4 hours after injection. Symptoms of marked depression, weakness, prostration and in some animals, labored respiration just before death were noted. Animals in which death was delayed for 24 hours or more generally showed no symptoms except slight transient depression soon after injection.

Occasionally animals receiving monacetin and diacetin exhibited local irritation of the skin at the site of injection. In the eye of the rabbit, 50% monacetin caused only a slight degree of transient injection of conjunctival vessels, but diacetin and triacetin in similar concentrations caused marked congestion and moderate edema.

The effect of the acetins on red blood corpuscles of the dog was studied. Dilutions of monacetin in 0.9% NaCl solution of from 1:500 to 1:4,000 did not cause hemolysis over 24 hours. At 18 hours slight hemolytic effects were noted with diacetin and triacetin in from 1:500 to 1:2,000 dilutions. Spectroscopic examination of these samples did not show the characteristic band for acid hematin.

Aqueous solutions of acetins were distinctly acid. The pH of solutions of 50% monacetin and of diacetin was 3.88 as determined with the quinhydrone electrode. In 1:14 dilution the pH for monacetin, diacetin and triacetin was 4.52, 4.18 and 3.88, respectively. Monacetin was miscible with an equal volume of dog's serum, while diacetin precipitated serum, triacetin and serum produced an emulsion. The pH of such serum mixtures was 7.45, 7.16 and 6.43, respectively as determined with the glass electrode.

Summary. A study of the toxicity of the acetins reveals that the subcutaneous LD_{50} for monacetin is approximately 5.5 cc per kilo for inbred albino rats, and 3.5 cc per kilo for inbred white mice. The LD_{50} dose for diacetin is 4.0 cc and 2.5 cc per kilo, respectively, and for triacetin 2.8 cc and 2.3 cc per kilo, respectively. Slight irritation to mucous membranes and occasionally to cutaneous tissues was noted with monacetin. Monacetin did not hemolize blood corpuscles in dilutions of 1:500 or greater. Blood serum and monacetin are compatible with a resulting pH of 7.45. Diacetin and triacetin are more irritating to tissues, are slightly hemolytic and are relatively incompatible with blood serum.

11877

Influence of Splenectomy on Susceptibility of Mice to Infection with *Taenia taeniaeformis* Eggs.

R. Hoeppli.

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The following experiment was carried out to determine if splenectomy would make mice more susceptible to infection with eggs of *Taenia taeniaeformis*, as it is well known that the removal of the spleen does lower the resistance of mice against various pathogenic organisms. Inbred mice* were used of the same comparatively resistant strain PCDM which had served in previous series of experiments, Feng and Hoeppli,[1] Fortuyn and Feng.[2] By the earlier experiments, it had been found that mice of the PCDM strain, compared with mice of the other inbred strains, had on the average a smaller number of cysts in the liver when fed with an equal number of eggs. There were, however, also in the PCDM strain always a few mice which became heavily infected whereas a few others remained completely negative, indicating that apart from a comparatively high resistance in the great majority of the PCDM animals there were a few individuals with either a complete or a very low resistance. Notwithstanding these few exceptions, the PCDM strain appeared on account of its comparatively higher resistance particularly suitable for the present experiment.

Material and Method. Altogether 56 PCDM mice of about the same age (3-4 months) were used. They were divided into 2 groups of 28 each and the mice of one group had their spleens removed under ether anaesthesia. Both groups were kept for observation for one month after the splenectomy had been performed. The blood of the 28 operated mice was examined at intervals but no organisms such as *Bartonella* or similar forms were found and the mice appeared not to suffer in consequence of the operation. As additional control of the viability of the *Taenia* eggs, one normal white laboratory bred rat about 3 months old was used. *Taenia* eggs were obtained from gravid segments of *T. taeniaeformis* from a freshly killed experimentally infected cat. As in the previous experiments, the eggs were administered by holding the experimental animal ab-

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* I wish to thank Dr. A. B. D. Fortuyn of the Department of Anatomy, P.U.M.C., for his kind coöperation in supplying the mice.

[1] Feng, L. C., and Hoeppli, R., *Chinese Med. J.*, 1939, **55**, 45.

[2] Fortuyn, A. B. D., and Feng, L. C., *Peking Nat. Hist. Bull.*, 1940, **15**, in press.

domen upward with its mouth forced open and by dropping the eggs suspended in normal saline from a glass dropper into the depth of the mouth cavity. The animal, as a rule, swallowed the fluid without objection.

Experiment. On July 10, 1940, the control rat, the 28 normal mice and the 28 splenectomized mice were infected each with 350-400 eggs. About 3 months later, on October 7th, all the animals were killed and the cysts in each liver examined and counted. The control rat was found to harbor 120 well developed cysts with living larvae. In both sets of mice, it was observed that only a comparatively small number of cysts corresponded in size to a normal 2-month-old cyst in mice and contained living larvae. The great majority consisted of either very small cysts or of merely small white spots which were solid and evidently represented the place where larvae had developed for a brief period. In Table I, the combined number of cysts and white spots is given for each mouse.

Result. In order to analyze the figures of the table, the following

TABLE I.
Combined Number of Cysts and White Spots for Each Mouse. In Parentheses the Number of White Spots Alone.

Splenectomized mice			Normal mice		
No.			No.		
6	(4)	F	5	(5)	M
7	(3)	F	5	(1)	F
7	(7)	M	14	(13)	F
10	(10)	F	4	(4)	F
9	(7)	F	1	(1)	M
3	(3)	M	4	(4)	M
5	(5)	F	2	(1)	M
5	(5)	F	4	(4)	F
4	(4)	F	7	(7)	F
4	(4)	F	3	(3)	F
3	(3)	F	0	(0)	M
5	(5)	F	2	(2)	F
7	(7)	F	0	(0)	F
0	(0)	M	2	(2)	F
1	(1)	M	3	(3)	M
7	(5)	F	1	(1)	M
7	(7)	M	3	(3)	F
6	(6)	F	2	(2)	F
13	(13)	M	4	(4)	M
9	(7)	F	3	(3)	M
0	(0)	F	4	(4)	F
0	(0)	M	1	(1)	F
0	(0)	M	3	(3)	F
4	(4)	F	0	(0)	M
0	(0)	F	8	(8)	M
3	(3)	M	10	(10)	M
9	(5)	F	18	(18)	M
14	(13)	M	3	(3)	M
148	(131)		116	(110)	

were considered: The total of cysts plus spots in each group; the total of cysts alone and of spots alone in each group; the average of cysts plus spots in each group. In addition, the method of pairing and the t-test was applied and also the z-test to determine the importance of sex.

Results. The total number of cysts plus spots is higher in splenectomized mice (148) than in normal ones (116). In splenectomized mice, 17 cysts developed to proper size (131 spots), as compared with only 6 cysts in the non-operated mice (110 spots). The average number of cysts plus spots in splenectomized mice is 5.28 ± 0.70, in normal mice 4.14 ± 0.76. This average number of cysts plus spots in the 2 groups does not differ significantly because the error attached to it is too great. When the t-test is applied, however, it becomes evident that the number of cysts found in splenectomized mice differs significantly from the number found in the normal group. This is especially true for individuals with medium resistance. When either no cysts plus spots or a large number are found, the difference between the 2 groups is not obvious. The z-test showed that sex differences are insignificant. It is evident that besides splenectomy, there are other unknown factors contributing to the variability of the number of cysts which are of equal or greater importance.

Conclusion. In inbred mice of a strain which is somewhat resistant to infection with eggs of *Taenia taeniaeformis,* splenectomy increases the susceptibility to infection except in those comparatively few mice with an individual resistance that is either abnormally high or abnormally low.

11878

Distribution of Sulfanilamide in the Ocular Fluids and Tissues After Local Application.

S. Y. P'AN. (Introduced by Hamilton H. Anderson.)

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Marshall[1, 2] first demonstrated that after oral administration,

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[1] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

[2] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. Pharm. and Exp. Therap.*, 1937, **61**, 196.

sulfanilamide is rapidly absorbed and is present in approximately equal concentrations in body tissues except bone and fat. Rambo,[3] Bellows and Chinn[4] and Mengel[5] have shown that the drug is present in the aqueous or vitreous humors or ocular tissues, after oral administration in animals or man. There are few reports on the absorption of sulfanilamide after local application into the conjunctival sac and the results reported are not uniform. Mengel[5] and Guyton[6] found that when a solution of sulfanilamide was applied into the conjunctival sac, concentrations of from less than 0.1 to 0.8 mg % were found in the aqueous humor. Bellows and Chinn[4] dusted sulfanilamide powder into the conjunctival sacs of dogs and allowed it to remain for 2 hours. They were unable to detect any trace of the drug in either the aqueous or vitreous humors. Lou and P'an,[7] on the other hand, found that sulfanilamide was present in significant amounts in the aqueous humor of human eyes after local instillation of a warmed 1% solution (concentration in aqueous humor varied from 1.0 to 4.2 mg %) or powdered sulfanilamide (concentration in aqueous humor varied from 4.5 to 44.4 mg %). The distribution of sulfanilamide in the various ocular tissues and fluids after local application has not hitherto been reported and is, therefore, the subject of this study.

Except in 2 rabbits in which sulfanilamide was applied to both eyes, 100 mg of finely divided sulfanilamide was applied to the conjunctival sac of one eye using the other eye as a control in each of the remaining 17 albino rabbits. Animals weighed from 1.5 to 2.2 kilos. The treated eye was kept closed by the application of a clamp. After an interval of one hour the animal was anesthetized with ether and samples of aqueous humor and blood were drawn. Both eyes, including the conjunctivae, were then removed and immediately dissected into the following components: conjunctiva, cornea, sclera, lens, vitreous humor and the chorioretinal layers. Tissues were washed three times in 0.9% NaCl solution, weighed and ground with sand and an appropriate amount of 5% para-toluene-sulfonic acid was added so that each cc contained from 10 to 50 mg of the tissue. These samples were centrifuged and filtered. To 2 cc of the filtrate was added 2 cc of water, and this was treated with sodium nitrite and dimethyl-alpha-napthalamine and compared with an appropriate standard according to the method of Marshall[8] in an Evelyn photoelectric colorimeter using filter No. 520. Samples of blood or aqueous humors were tested by adding 0.1 cc of the sample to 5 cc of water and precipitating with 2 cc of 15% para-toluene-sulfonic acid. This was filtered and 4 cc of the filtrate was used for the determination. Duplicate analyses were made whenever adequate samples were available.

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[3] Rambo, V. C., *Am. J. Ophth.*, 1938, **21**, 739.

[4] Bellows, J. G., and Chinn, H., *J. A. M. A.*, 1939, **112**, 2023.

[5] Mengel, W. G., *Arch. Ophth.*, 1939, **22**, 406.

[6] Guyton, J. S., *Am. J. Ophth.*, 1930, **22**, 833.

[7] Lou, T. H., and P'an, S. Y., *Chin. M. J.*, 1940, **58**, 167.

[8] Marshall, E. K., Jr., *J. Biol. Chem.*, 1937, **122**, 263.

Half of the tests were made according to the micromethod of MacLachlan *et al.*[9] using 0.1 cc of fluid or blood. The ocular tissues were ground with sand and 5% para-toluene-sulfonic acid was added as described above, 2 cc of the filtrate being used in these tests, comparisons being made with an appropriate standard in a Hasting's bicolorimeter.* The third washings of the various tissues were examined for the presence of sulfanilamide and found to be negative.

The mean concentrations of free sulfanilamide found in the ocular fluids and tissues of the 19 rabbits tested after local application of the drug are, in their decreasing order as follows: Cornea—185.4±13.2; conjunctiva—89.8±14.0; aqueous humor—54.8±5.3; sclera—23.3±4.6; chorioretinal tissue—12.3±1.2; lens—8.7±1.8; and vitreous humor—1.1±0.2 mg %.

No trace of sulfanilamide was found in the ocular fluids and tissues of untreated eyes, or in the blood, except in 2 instances where 1.8 and 1.9 mg % was detected. Except in the vitreous humor, lens and chorioretinal tissues the concentrations of sulfanilamide in the ocular fluids and tissues are higher than those obtained after the oral administration of moderate amounts of the drug in man and rabbits.[3, 5, 6, 7, 10]

Local application of powdered sulfanilamide into the conjunctival sac would appear to be the method of choice for obtaining adequate concentrations of the drug in ocular tissues and fluids, since tremendous doses would be required orally or parenterally to maintain similar levels with the consequent danger of untoward effects. Sulfanilamide has had some effect in controlling certain infections limited to the ocular region, especially trachoma and gonoblennorrhea.

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[9] MacLachlan, E. A., Carey, B. W., Jr., and Butler, A. M., *J. Lab. Clin. Med.*, 1938, **23**. 1273.

* Ten duplicate tests were made with each colorimeter on samples of rabbit's blood to which sulfanilamide was added to make concentrations of 50 and 100 mg %. Similar results were obtained with each colorimeter, within 4 % of the known amount at 50 mg % and within approximately 5.5 % of the known amount at 100 mg %.

[10] Long, P. E., and Bliss, E. A., *The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine and Allied Compounds*, New York, The Macmillan Co., 1939, pp. 151-153.

When such diseases are localized local application of the drug in powdered form would appear to be preferable to other methods of administration where general systemic absorption is not essential, especially in hypersensitive patients.

In rabbits a slight to moderate congestion of the conjunctiva was observed after sulfanilamide had been applied locally. Microscopic examination of the cornea removed from four of the treated eyes showed no significant changes while the conjunctiva exhibited a moderate degree of desquamation and a very slight leucocytic infiltration just beneath the epithelium. These changes may be due to the mechanical rather than the chemical effects of the powdered drug.†

Summary. Sulfanilamide was found to be present in significant concentrations in ocular tissues and fluids after its local application in powdered form into the conjunctival sacs of rabbits. No appreciable untoward reactions were encountered in the 19 rabbits studied. Local application of the drug for 1 hour did not result in an appreciable systemic absorption, since no sulfanilamide could be detected in blood samples, with two exceptions, or in ocular tissues and fluids of the untreated eyes of test animals.

11879 P

Specific Polysaccharide as Cutaneous Test for Evaluation of Serum Therapy in Influenza Bacillus Meningitis.

JOHN H. DINGLE AND LEAH R. SEIDMAN.
(Introduced by J. F. Enders.)

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The use of specific polysaccharides injected intracutaneously as a means of evaluating the adequacy of serotherapy was first advocated in pneumonia by Francis,[1] and has since been employed by others[2, 3, 4] with variable results. The immediate wheal and erythema which characterize the positive reaction are apparently due to an antigen-antibody reaction at the site of injection and are probably dependent chiefly on an excess of circulating antibody. This reports deals with preliminary experiments which indicate that a similar immediate reaction may be obtained with specific polysaccharides following serotherapy in patients suffering from meningitis due to *Hemophilus influenzae*, type *b*, and that the test constitutes a simple method of determining the presence of an excess of antibody in the blood stream.

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† The author is indebted to Dr. C. H. Hu for examining the histological sections and to Dr. S. H. Liu for the use of the Evelyn photoelectric colorimeter.

[1] Francis, T., Jr., *J. Exp. Med.*, 1933, **57**, 617.

[2] Finland, M., and Brown, J. W., *J. Clin. Invest.*, 1938, **17**, 479.

[3] Felton, L. D., and Prather, P. F., *Pub. Health Rep., U.S.P.H.S.*, 1939, **54**, 1053.

[4] Wood, W. B., Jr., *J. Clin. Invest.*, 1940, **19**, 95, 105.

The test consisted of the intracutaneous injection of 0.01 mg of polysaccharide isolated from *H. influenzae*, type *b*[5] in 0.1 ml of physiological salt solution (1-10,000 dilution). An equal amount of saline solution injected into the skin of the same or opposite forearm served as a control. Readings were made at 20 minutes (immediate reaction) and at 24 hours (delayed reaction). Blood specimens taken before serum therapy and at the time of the injections were examined for the presence of antibodies by one or more of the following tests: precipitation with homologous polysaccharide, bactericidal action,[6] or capsular swelling[7] (Neufeld reaction). The sera employed in therapy were anti-*H. influenzae* horse serum obtained from the Massachusetts State Antitoxin Laboratory and anti-*H. influenzae* rabbit serum prepared by us and by Dr. J. A. V. Davies of the Infants' and Children's Hospitals.

Seven patients treated intravenously and intrathecally with horse antiserum were tested. In no instance did the immediate reaction show definite wheals in spite of a demonstrable excess of antibody. In only 2 patients was an erythematous zone larger than 10 mm demonstrable. No delayed reactions occurred.

With rabbit antiserum, on the other hand, marked immediate reactions occurred in 5 patients within 2 to 8 hours after intravenous administration of the serum, provided that an excess of antibody was present. No tests were made less than 2 hours after serotherapy. The reactions consisted of an area of erythema varying up to 50 mm in diameter with a central wheal, 10 to 15 mm in diameter, showing variable numbers of pseudopodia. The test remained positive in 2 patients who recovered and in whose blood antibody was demonstrable by other methods. Further study is necessary to correlate the duration of antibody excess and positive skin reactions. No delayed reactions were noted.

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[5] Dingle, J. H., and Fothergill, L. D., *J. Immunol.*, 1939, **37**, 53.

[6] Dingle, J. H., Fothergill, L. D., and Chandler, C. A., *J. Immunol.*, 1938, **34**, 357.

[7] Alexander, H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 313.

One uninfected subject failed to react to the polysaccharide either before or after the intravenous administration of horse antiserum. Two weeks later the reaction was still negative. She was then given anti-*H. influenzae* rabbit antiserum intravenously. Subsequent intracutaneous injection of the polysaccharide gave a marked immediate reaction. All patients prior to serotherapy, and all uninfected controls in the same age groups failed to react to the test. It has been demonstrated[8] that the blood of normal adults contains bactericidal antibodies for this organism. Ten such normal adults showed negative skin reactions to the polysaccharide. Further work is necessary to determine adequately the specificity and sensitivity of this reaction.

Summary. Positive immediate skin reactions to the specific polysaccharide may be obtained in patients with influenza bacillus meningitis and in normal individuals following the administration of anti-*H. influenzae* rabbit serum in amounts sufficient to establish an excess antibody concentration in the blood. The test is proposed for further investigation as a simple clinical method of evaluating the adequacy of serotherapy.

The authors gratefully acknowledge the suggestions and encouragement of Drs. J. A. V. Davies and L. D. Fothergill.

11880 P

Toxicity and Fate of the Lethal Effect of Theophyllinated Scillaridin* in the Cat.

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Riseman and London[1] have recently described some of the properties of a substance which is obtained by condensing a disodium salt of a mixture of genins isolated from Squills (presumably scillaridin A and B) with 2 molecules of theophylline. They demonstrated by electrocardiographic studies that the substance so obtained differs in its action from either a mechanical mixture of theophylline with the genins, or from the original glycosides, thus giving physiological confirmation for a discrete compound. Since this appears to be the first report of an attempt to modify the action of a cardiac glycoside by chemical combination with a xanthine derivative, it seemed to be of considerable interest to determine the toxicity of the substance and the fate of the lethal effect of sublethal doses in the cat. The ratio of genins present in the compound has not been determined, but the empirical formula may be approximated as $C_{24}H_{28}O_3 \cdot (C_7H_7O_2N_4)_2 \cdot 2H_2O$. It should be pointed out however, that by the method of preparation used, structural rearrangement of the genin may well have taken place. The influence of structure on physiological action is discussed further by Lendle.[2]

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[8] Fothergill, L. D., and Wright, J., *J. Immunol.*, 1933, **24**, 273.

* Supplied by Parker Dorn, Inc., Worcester, Mass.

[1] Riseman, J. E. F., and London, S. B., *Science*, 1940, **92**, 384.

The preparation was assayed by the original Hatcher and Brody procedure using a 1:714 solution in normal saline and a rate of infusion such that the animals died within 30 to 90 minutes. The geometric mean lethal dose on the basis of 10 cats was found to be 21.4 mg per kg (or 28.2 micromoles per kg) and the standard deviation was 4.2. By comparison, the lethal doses[3] of Scillaridin A and of Scillarin A are respectively 0.150 and 1.72 mg per kg (0.217 and 4.73 micromoles per kg). The toxicity of the substance is thus much less than that of two of the substances related to it chemically. Calculation of the lethal doses in micromoles per kg shows that this decrease in toxicity cannot be explained on the basis of difference in molecular weight.

The fate of the lethal effect of sublethal doses was determined by the procedure described previously.[4] A preliminary injection of 13 mg per kg (60% of the lethal dose) of theophyllinated Scillaridin (1.3%) was given intravenously to each of 31 cats. These were divided into 3 approximately equal groups and sacrificed as follows: the first group was killed 3 hours after injection by an infusion of ouabain (1:200,000) at one to 2 cc per minute. The second group was also killed 3 hours after injection, but with the preparation itself (1:714) and the third group received the theophyllinated Scillaridin (1:714) infusion 24 hours after the initial injection. The results are given in the table. The geometric mean lethal dose of ouabain was previously reported to be 0.10 mg per kg.[4]

It will be seen from the table that 3 hours after administration

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[2] Lendle, L., *Handbuch der Experimentellen Pharmakologie*, supplementary v. 1, Springer, Berlin, 1935, p. 77.

[3] Chen, K. K., Robbins, E. B., and Worth, H., *J. Am. Pharm. Assn.*, 1938, **27**, 189.

[4] DeGraff, A. C., and Lehman, R. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 323.

TABLE I.
Dissipation of the Lethal Effect After 3 and 24 Hours.

Hrs	No. of animals	Mean fraction of lethal dose required in final infusion	σ	P	% dissipation of initial dose
3	10	0.76 (Ouabain)	0.139	<0.01	60
3	9	0.79 (Theophyllinated Scillaridin)	0.208	<0.05	65
24	12	0.77 (Theophyllinated Scillaridin)	0.112	<0.01	62

of 60% of a lethal dose of the new compound, the lethal dose of ouabain has fallen to an average of 76% of its original value. Essentially the same result was obtained when theophyllinated Scillaridin was used after 3 and 24 hours, that is, 79 and 77% of the mean lethal dose respectively was required. The significance of the differences between these mean values and the means for the assays of ouabain and theophyllinated Scillaridin was determined by calculating the statistics s, t, and P according to Fisher's notation.[5]

Thus, P in the table gives the probability that the assay values obtained with and without premedication are drawn from the same population. P is found to lie well below 0.05 and therefore, the means listed in the table may be said to differ significantly from the corresponding mean results of bioassay. In the last column of the table the data have been calculated in terms of the initial dose and are expressed as percent dissipation as follows:

$$\frac{100\ (0.60 + \text{final dose} - 1)}{0.60}$$

where the final dose is expressed as a fraction of the Hatcher and Brody lethal dose.

Since the same percentage dissipation was found for theophyllinated Scillaridin when the titration was done with ouabain as with the drug itself, it is probable that the lethal action of these two drugs is qualitatively similar. It may also be concluded by comparison with the results on the lanata glycosides[4] that the rate of dissipation is relatively rapid, but does not resemble that of digoxigenin as might have been expected. It would seem, then, that substitution of xanthine for carbohydrate in the squills glycoside molecule lowers the toxicity but does not remove the so-called "cumulative" property.

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[5] Fisher, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 7th ed., 1938, p. 128.

11881 P

Resting Blood Flow in the Extremities in Hyperthyroid Subjects.*

David I. Abramson and Sidney M. Fierst.

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In view of the flushed, moist, warm skin, the wide pulse pressure, and the decrease in circulation time present in thyrotoxicosis, it has generally been assumed that there is an increase in blood flow to the periphery in this state. The recent findings of Stewart and Evans[1] of an increased skin temperature, as determined from the rate of heat radiation, are in accord with this concept. Since all of the above evidence is indirect, it was thought worthwhile to reëxamine the subject by studying the actual rate of blood flow to the hand and forearm, using the venous occlusion plethysmographic method.

Five hyperthyroid patients were examined, and in 3, blood flow readings were obtained before and after thyroidectomy. For contrast, the results in a case of moderate hypothyroidism were also included in the study. The exact technique used in determining the rate of blood flow is described in detail elsewhere.[2] The bath temperature in the plethysmograph was maintained at 32°C and the subject was exposed to the ordinary room temperature. Ten readings were obtained in each experiment and from the average of these, the rate of blood flow per minute per 100 cc of limb volume was calculated. The blood pressure, pulse rate, circulation time (arm to tongue), and the basal metabolic rate were also determined.

It is obvious from Table 1 that in every case of hyperthyroidism there was a significant increase in the rate of blood flow to the forearm, since the average normal level under similar conditions was found to be only 1.8±0.64 cc per minute per 100 cc limb volume.[3] In contrast, the forearm blood flow readings in the hypothyroid patient (with a basal metabolic rate of —20) was 0.8 cc per minute per 100 cc limb volume. Following lugolization and thyroidectomy, there was a definite decrease in flow in the hyperthyroid subjects.

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* Aided by the Samuel and Regina Kuhn Fund, and a grant from the Dazian Foundation for Medical Research.

[1] Stewart, H. J., and Evans, W. F., *Am. Heart J.*, 1940, **20**, 715.

[2] *a.* Abramson, D. I., Zazeela, H., and Marrus, J., *Am. Heart J.*, 1939, **17**, 194, 206; *b.* Ferris, E. B., Jr., and Abramson, D. I., *Am. Heart J.*, 1940, **19**, 233.

[3] Abramson, D. I., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 127.

TABLE I.
Resting Blood Flow to the Extremities in Hyperthyroidism.

Patient	Date	B.P.	Pulse per min.	Circulation time, sec.	B.M.R.	Blood flow, hand	Blood flow, forearm	Period of lugolization, days	Remarks
M.B.	8/1	$\frac{124}{60}$	106	9	+56 +59	11.1	4.7		preoperative
	8	$\frac{128}{64}$	110	9	+53 +49		4.7	1	”
	27	$\frac{106}{60}$	85	10		9.8	2.9		7 days postoperative
	9/24	$\frac{110}{68}$	86	10	— 2 0	11.4	1.0		35 days postoperative
J.L.	7/6	$\frac{130}{88}$	92	11	+37 +42	5.1	6.8	3	preoperative
	17	$\frac{136}{88}$	78	11.5	+19 +24	6.4	3.9	14	”
	29	$\frac{142}{90}$	77	13	+12 + 9	10.3	3.3		10 days postoperative
	9/25	$\frac{138}{80}$	80	12	+16 +18	8.1	1.4		68 days postoperative
S.S.	2/27	$\frac{132}{88}$	134	6-7	+65 +70	13.0	3.8		preoperative
	4/7	$\frac{116}{78}$	74		+ 3 + 1	5.4	0.9		11 days postoperative
E.H.	2/15	$\frac{154}{84}$	83		+61	9.2	4.4		
A.E.	11/19	$\frac{116}{66}$	96	10.5	+23	2.4	3.2		

Blood flow expressed in cc per min. per 100 cc limb vol.

with a return to the normal level within 11 to 68 days after operation.

In the hand, the blood flow in the hyperthyroid cases was not increased beyond the average normal level of 10.1 ± 3.4 cc per minute per 100 cc limb volume.[4] Following thyroidectomy there was no significant change in 2 cases, while in one there was a drop from a high normal to a lower normal level. In the hypothyroid subject, the blood flow in the hand was 4.4 cc.

On comparing the blood flow readings with the other data collected at the same time, it was noted that a gross correlation existed between the blood flow to the forearm and the basal metabolic rate, but none between the latter and the blood flow to the hand. No relationship was observed between the circulation time and the blood flow in either the hand or forearm.

In view of the increased cardiac output[5] and increased blood volume[6] found in hyperthyroidism, an augmented rate of blood flow to the extremities might be anticipated. The fact that this was observed only in the forearm and not in the hand would suggest that there is some other factor, possibly related to the intrinsic differences between these 2 vascular beds,[7] which is operating in the hand to modify the vascular responses associated with the hyperthyroid state.

Conclusion. The rate of blood flow through the forearm is increased in hyperthyroidism.

11882 P

Regeneration of Olfactory Cells.*

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It is generally held that nerve cells which have been destroyed are not restored. While this may well be true for the type of nerve cells which are usually considered in this connection, it has not been demonstrated to apply to the neurons of the olfactory nerve, the cell

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[4] Abramson, D. I., unpublished observations.

[5] *a.* Grollman, A., *The Cardiac Output in Health and Disease*, Charles C. Thomas, 1932, p. 242; *b.* Fullerton, C. W., and Harrop, G. A., Jr., *Bull. Johns Hopkins Hosp.*, 1930, **46**, 203; *c.* Boothby, W. M., and Rynearson, E. H., *Arch. Int. Med.*, 1935, **55**, 546.

[6] *a.* Gibson, J. G., 2nd, and Harris, A. W., *J. Clin. Invest.*, 1939, **18**, 59; *b.* Goldbloom, A. A., and Libin, I., *Arch. Int. Med.*, 1935, **55**, 484.

[7] Abramson, D. I., and Ferris, E. B., Jr., *Am. Heart J.*, 1940, **19**, 541.

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

bodies of which retain the primitive location in the surface epithelium found in certain lower forms of animal life (neuroepithelium). The cell bodies of the olfactory nerve (olfactory cells) are located for the most part in the mid-zone of the fairly thick pseudostratified columnar epithelium of the olfactory mucosa.[1] Each cell gives off two processes: (1) a superficial stout one, or dendrite, which extends slightly beyond the surface of the mucosa and (2) a deep one, or axone, which passes centralward to the olfactory bulbs under the frontal lobe of the brain. These processes can be demonstrated only by special staining procedures, such as the Bodian method for showing neurofibrils,[2] and are less easily stained than the fibrils of other nerves.

In previous communications[3] we have reported that it is possible to induce a high degree of resistance in monkeys against intranasal inoculation with the virus of poliomyelitis by a previous irrigation of the nasal passages with a 1% solution of zinc sulfate. The resistance so induced generally lasts at least one month, but is almost without exception followed by a return of susceptibility within the next 3 or 4 months. It is initiated by an extensive coagulation necrosis of the olfactory mucosa, followed usually within a day or two by its separation, more or less en masse, from the underlying lamina propria and adjacent glands of Bowman.[1] Since the olfactory cells occur chiefly in the mid-zone of the olfactory epithelium it is obvious that they must be cast off with the necrotized epithelium. If the generally accepted view regarding the failure of nerve cells to regenerate applies to olfactory cells also, these should then be lost permanently and not appear in the new epithelium.

Following the separation of the damaged epithelium from the lamina propria general epithelial repair takes place rapidly. The cells which participate in this re-epithelization seem to come largely from the ducts and glands of Bowman adjacent to the denuded surface; in part also from the Schwann cells of nearby olfactory nerve bundles. The new epithelium usually attains the thickness of the normal within the space of a few days, but abnormalities in cell arrangement make it for a time distinguishable from normal mucosa. It is not the purpose of this communication to describe the general re-epithelization, nor the features of the new epithelium, but to report observations indicating that destroyed olfactory cells also are restored, at least partially, following this re-epithelization. This seems evident from an examination of several thousand specially stained histological sections of olfactory mucosae, obtained from more than 200 monkeys previously treated with zinc sulfate (or with other necrotizing agents), the animals having been sacrificed at varying intervals of time, from one day to more than 6 months after one or more intranasal irrigations with the chemical solution. The nature of our observations can be indicated only briefly and include: (1) the presence in about 2 weeks of cells, or nests of cells, resembling olfactory cells, but apparently without dendritic and axonal processes, along with similar cells seemingly in the act of forming such processes; (2) abnormalities in the form, orientation, grouping and distribution of olfactory cells in later stages, together with unusual prominence of axones and olfactory nerve bundles in the lamina propria in association with such cells in late membranes; (3) the extent of mucosa which after some months shows a varying number of olfactory cells, compared with the extent of the initial destruction usually seen within 2 or 3 days after applying the solution.

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[1] Schaeffer, J. P., *The Nose and Olfactory Organ*, Phila., 1920, Blakiston; *Special Cytology*, Ed. by E. V. Cowdry, Hoeber, 1928, I, 47.

[2] Bodian, D., *Anat. Rec.*, 1936, **65**, 89.

[3] Schultz, E. W., and Gebhardt, L. P., *J. Am. Med. Assn.*, 1937, **108**, 2182; Schultz, E. W., *Am. J. Surg.*, 1938, **42**, 178; *Am. J. Pub. Health*, 1940, **30**, 175.

Apparently such restoration as is effected is seldom complete with relation to the total number of cells. In some cases it seems to have been extensive, in others only slight. Certain factors which may influence the degree of restitution are under consideration. Obviously, evidence of the extent of the regeneration, even more than the fact of regeneration itself must be largely circumstantial for it is quite impossible ever to be certain of the actual extent of the initial destruction.

Regarding the origin of the new olfactory cells, we can at present only state that their progenitors seem to share in the initial general epithelization.

Further studies are in progress to rule out other explanations for the observations made.

11883 P

Effects of Hypophysectomy on Some Renal Functions.*

H. L. WHITE, PETER HEINBECKER AND DORIS ROLF.

From the Departments of Physiology and of Surgery, Washington University School of Medicine, St. Louis.

We have reported[1] that diodrast (D) and inulin plasma clearances are markedly reduced in hypophysectomized dogs. It was not determined whether the low D clearance was due to a low renal plasma flow (RPF) or to a diminished renal extraction of D, or to both. The present paper reports on the effects of "simple" hypophysectomy (section of stalk with removal of dependent gland) and of complete hypophysectomy (includes destruction of median eminence) on D plasma clearance, plasma D extraction, RPF, tubular extraction of D, maximum tubular excretory rate for D (D Tm), inulin clearance, filtration fraction and blood volume. It also reports on the question of glomerular intermittence after hypophysectomy, with the finding that intermittence does not occur. Since no certain difference in effects of simple and of complete hypophysectomy was observed on any of the processes studied, no distinction will be made in the present report; the effects are apparently due to loss of anterior lobe, although further observations are required before a final statement can be made. Six dogs have been studied; pre-hypophysectomy observations of most of the processes studied were made on the same animals.

Diodrast plasma clearance. In 15 post-hypophysectomy clearance periods on 3 dogs (K6, K10 and K11) taken at 7 to 93 days after operation, D plasma clearance has averaged 141 cc/min./M^2, where the normal was 258. The clearance of one completely hypophysectomized dog (K8) on the 4th postoperative day was 155, normal 222; a "simple hypophysectomy" dog (K9) on the 3rd day showed 191 cc/min./M^2, where normal had been 208. One dog (K1), with a normal clearance of 232, had a unilateral nephrectomy on 7/22/40 and a simple hypophysectomy on 7/26/40; on 9/4/40 her D clearance was 107. The kidney removed on 7/22/40 weighed 54 g, the remaining kidney at death on 9/30/40 weighed 52 g. These observations indicate that while hypophysectomy prevents renal hypertrophy it does not prevent a maintenance of normal D clearance per gram of kidney in the remaining kidney after uninephrectomy, *i.e.*, clear-

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* Aided by a grant from the Commonwealth Fund to H. L. White.

[1] White, H. L., and Heinbecker, P., *Am. J. Physiol.*, 1940, **130**, 464.

ance through remaining kidney was approximately half that of both kidneys while normal.

Plasma D extraction. Plasma D extraction has not been changed from normal by hypophysectomy, the values ranging from 0.71 to 0.85. While a number of post-hypophysectomy renal vein samples have shown anomalously low extractions, the probability of this has not been higher than in normals; an extraction less than 0.7 has been arbitrarily called anomalously low.

Renal plasma flow. RPF was determined as inulin plasma clearance/inulin plasma extraction and as D plasma clearance/plasma D extraction × 1.12 (12% of the D output is contributed by cells); the 2 methods usually agree within 8% and practically always within 10%. A completely hypophysectomized dog (K10), with a normal RPF of 374 cc/min./M^2, showed RPF of 224 on the 20th postoperative day. A simple hypophysectomy dog (K11), with a normal RPF of 268, showed 162 on the 7th postoperative day. If our present belief, that plasma D extraction is not altered by hypophysectomy, continues to be sustained on subsequent observations, it will then be permissible to determine RPF as D plasma clearance × 1.2 on dogs without renal explants, hypophysectomized as well as normal.

Tubular extraction of D. If the term "tubular extraction"[2] is modified to read

$$\frac{\text{tubular clearance of D}}{\text{RPF} - \text{glomerular clearance of D}}$$

it will give a better expression of the tubules' effectiveness in extracting D than does the original expression, tubular clearance of D/RPF. The new expression tells what fraction of the D presented to the tubules is extracted by the tubules, while the original expression tells what fraction of the D presented to the kidneys is extracted by the tubules. The former averaged 0.77 in 2 dogs (K10 and K11) while normal and 0.79 after hypophysectomy, indicating that the effectiveness of the tubules in extracting D at low rates is not affected by hypophysectomy.

Diodrast Tm. Diodrast Tm has averaged 18 mg iodine/min./M^2 on normals and 8 mg/min./M^2 at 7 to 26 days after hypophysectomy. In one experiment (K10) on the 26th postoperative day 3 Tm periods were run at a plasma I level of 23 mg %, with an average tubular output of 8.1 mg iodine/min./M^2; the next three periods were run at a plasma I level of 39 mg %, with an average tubular output of 7.7 mg/min./M^2. These findings show that the maximum tubular excretory rate for D is significantly diminished by hypophysectomy and are in contrast with the absence of effect on tubular extraction of D at low rates of output.

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[2] White, H. L., *Am. J. Physiol.*, 1940, **130**, 582.

Inulin clearance and filtration fraction. In 3 dogs (K9, K10 and K11) while normal, inulin clearance has averaged 84 cc/min./M^2 and filtration fraction (average of inulin clearance/D RPF and of inulin extraction) 0.27; at 3 to 26 days after hypophysectomy inulin clearance has averaged 55 and filtration fraction 0.26. The drop in glomerular filtration rate parallels the drop in RPF.

Blood volume. Plasma volumes were determined essentially according to Gibson and Evelyn.[3] Plasma and blood volumes in normals have averaged 60 and 115 cc/kilo, respectively; in hypophysectomized dogs they have averaged 48 and 92 cc.

Discussion. The present findings indicate that the low D plasma clearance of hypophysectomized dogs means a low RPF rather than a low extraction of D. A low RPF would not, however, explain a low D Tm, which should remain normal even with a low RPF, provided sufficient D is presented to the tubules, if the normal excretory capacity of the tubules for D is maintained. That sufficient D is being presented to the tubules in these experiments is proved by the experiment on K10 cited under Diodrast Tm, in which the rate of tubular output of D was not increased on raising the plasma I level from 23 to 39 mg %.

Two possible explanations of the low D Tm suggest themselves. The first is that, on the average, fewer nephrons are being supplied with blood in the hypophysectomized than in the normal animal (glomerular intermittence). This possibility appears to be excluded by the finding that the picture of ink-injected kidneys of 3 hypophysectomized dogs was the same as that in normal dogs, which seems best explained on the view that all the glomeruli are open all the time.[4] The second possibility is that the tubular mechanism for excreting D is deficient in hypophysectomized dogs, and this seems to be the correct one. Since the tubular extraction of D at low plasma levels is not affected, the findings may be interpreted as evidence that some hypothetical substance concerned in the tubular excretion of D[5] is deficient in quantity but is of normal quality. Further observations will reveal whether D Tm returns toward normal late after hypophysectomy.

Since the fall in RPF occurs with an unchanged filtration fraction and in the absence of glomerular intermittence, the renal vasoconstriction appears to involve all parts of the renal vascular tree uniformly. The arterial blood pressure in these animals is only slightly lowered. The fall in blood volume, which is probably accompanied by a fall in cardiac output, also must be partially responsible for the fall in RPF.

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[3] Gibson, J. G., 2nd, and Evelyn, K. A., *J. Clin. Invest.*, 1938, **17**, 153.

[4] White, H. L., *Am. J. Physiol.*, 1939, **128**, 159.

[5] Shannon, J. A., *Physiol. Rev.*, 1939, **19**, 63.

11884

Technic for Demonstrating Antibodies Against Tuberculin in Experimental Animals with Sensitized Collodion Pellets.*

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A method for utilizing suspensions of sensitized collodion pellets for determining the presence of specific antibodies in blood sera has recently been described by Cannon and Marshall.[1] Such a procedure appeared to offer a technic for studying the development of antibodies against tuberculin in tuberculous infection and in experimental procedures in which killed tubercle bacilli were used for immunization. It is the purpose of this paper to report the results obtained in testing the blood sera of such experimental animals with suspensions of collodion pellets sensitized with commercial old tuberculin.

Following the method described by Cannon and Marshall[1] a supply of collodion pellets was prepared. A suspension of these pellets having a turbidity-factor of 244 (Summerson-Klett photoelectric colorimeter with a No. 66 filter) was mixed with an equal amount of a 5% (by volume) solution of commercial old tuberculin. The mixture was allowed to stand overnight at a temperature of 4°C. The pellets were recovered and washed in double-distilled water by centrifuging and resuspending them 3 times. The final suspension of sensitized pellets was diluted to equal a standard suspension having a turbidity-factor of 154. Serial dilutions of the serum to be tested were made in saline solution so that the volume in each tube was 0.5 cc. To each dilution was added 0.5 cc of the suspension of sensitized pellets. After standing at room temperature for 20 minutes, the serum-pellet mixtures were centrifuged for 4 minutes at 1200 rpm. The result of each test was read after gently resuspending the pellets in each tube. The highest dilution of each serum causing agglutination of the pellets was recorded as the antibody-titer of that serum. Control tests of sensitized pellets in saline solution and of unsensitized pellets in dilutions of the serum being tested were made in the same manner.

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* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation in coöperation with the Jamaican Government.

[1] Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1940, **38**, 365.

To determine that suspensions of sensitized collodion pellets would accurately demonstrate the presence of antibodies against tuberculin in the blood serum of animals inoculated with tubercle bacilli, the following experiment was done. Twelve normal rabbits were bled from the marginal ear vein before inoculation. Six of the rabbits

TABLE I.
Results of Tests on Rabbits before and at Weekly Intervals after Vaccination with Heat-Killed Tubercle Bacilli.

	Before vaccination			1 wk after vaccination		
No. of animal	Pellet titer	Complement-fixation titer	Reaction to tuberculin	Pellet titer	Complement-fixation titer	Reaction to tuberculin
1	0	0	0	1-16	0	12x10x0.5
2	0	0	0	1-16	0	0
3	0	0	0	1-32	0	14x12x0.5
4	0	0	0	1-8	0	22x16x1
5	0	0	0	1-16	0	0
6	0	0	0	1-32	0	0
7	0	0	0	1-16	0	0
8	0	0	0	1-32	0	0
9	0	0	0	1-8	0	0
10	0	0	0	1-16	0	12x10x0.5
11	0	0	0	1-32	0	22x14x0.5
12	0	0	0	1-8	0	0

	2 wks after vaccination			3 wks after vaccination		
No. of animal	Pellet titer	Complement-fixation titer	Reaction to tuberculin	Pellet titer	Complement-fixation titer	Reaction to tuberculin
1	1-128	0	6x6x0.5	1-256	1-5	22x18x1
2	1-128	0	38x22x1	1-256	1-10	35x28x1
3	1-64	1-5	24x22x1	1-128	1-5	40x35x3
4	1-32	1-40	25x22x0.5	1-256	1-20	25x25x0.5
5	1-64	1-5	18x18x0.5	1-128	1-10	0
6	1-64	1-20	0	1-256	1-40	38x32x2
7	1-64	1-5	0	1-128	0	0
8	1-64	0	9x9x0.5	1-128	1-10	16x16x0.5
9	1-32	0	37x25x1	1-256	1-10	32x25x1
10	1-32	0	16x16x0.5	1-128	0	24x24x1
11	1-64	0	0	1-256	1-5	0
12	1-64	0	0	1-128	0	0

Note: Results of tuberculin skin tests represent diameter and depth of edema and hyperemia in millimeters present 48 hr after injection of 0.1 mg O. T.

(Nos. 7 to 12, Table I) received intracutaneous injections in 12 sites of 0.4 mg of heat-killed tubercle bacilli (Ravenel) suspended in saline solution. The remaining 6 animals (Nos. 1 to 6) were inoculated subcutaneously in 12 sites with 0.4 mg of the same preparation of tubercle bacilli, suspended in paraffin oil. Blood samples were obtained from the marginal ear vein at weekly intervals after inoculation for a period of 3 weeks. At the time each blood sample was taken, the animals were tested for skin-sensitivity to tuberculin by intracutaneous injection of 0.1 mg of old tuberculin. In addition to the tests with sensitized pellets, each serum was tested for complement-fixing antibodies, using heat-killed tubercle bacilli as an antigen. All tests were done at the conclusion of the experiment, so that all sera were tested with the same suspension of sensitized pellets. The results are recorded in Table I.

It will be noted in Table I that suspensions of collodion pellets sensitized with tuberculin uniformly demonstrate the presence of antibodies against tuberculin in the blood serum of the inoculated animals. All of the preinoculation sera failed to cause agglutination of the sensitized pellets, as did control tests with saline and unsensitized pellets. The method accurately indicates a rise in antibody production with increasing intervals of time after vaccination. It is interesting to note that the animals exhibiting a low degree of antibody production in the first postvaccination sera continue at a level lower than the other animals. The presence of antibodies against tuberculin is demonstrated by the use of sensitized pellets in sera, in which complement-fixing antibodies are not demonstrable and, in some instances, when skin-sensitivity to tuberculin is absent. There was no demonstrable difference in the degree of antibody production between the animals injected intracutaneously with saline suspensions of the tubercle bacilli and those injected subcutaneously with suspensions in paraffin oil.

The previous experiment indicates that suspensions of sensitized pellets differentiate between pre- and postvaccination blood sera. To check this observation further, 90 sera from other laboratory animals were obtained. These sera were tested with suspensions of sensitized pellets without knowledge of the previous history of exposure of the animals to tubercle bacilli. Tests for complement-fixing antibodies and tests for skin-sensitivity to tuberculin had been carried out at the time the bleedings were done. After the completion of the pellet tests, the animal records were consulted, and it was learned that 12 of the sera had come from animals which had never had a known exposure to tubercle bacilli. The remaining 78 sera were

TABLE II.
A Comparison of Tests on Inoculated and Normal Animals with Sensitized Collodion Pellets, Complement-Fixing Antigen, and Intracutaneous Tuberculin.

Type of tuberculous material inoculated	Animal	Sol. used to suspend inoculum	No. animals tested	No. of sera exhibiting positive reaction with sensitized pellets	No. of sera exhibiting positive complement fixation	No. of animals exhibiting positive tuberculin test
B. C. G.	Rabbit	Saline	5	5	2	4
Living Ravenel	"	"	2	2	1	2
Dry Jamaica 22	"	"	1	1	0	0
Heat-killed Ravenel	"	"	13	13	8	9
Dry Jamaica 22	"	Oil	17	17	8	7
Heat-killed Ravenel	"	"	33	33	23	21
Phosphatide Extract of Bacillus	"	Saline	5	5	3	0
Heat-killed Ravenel	Monkey	"	1	1	0	0
Heat-killed Ravenel	Baboon	"	1	1	1	1
None (Controls)	Rabbit	—	12	0	0	0

Note: The inoculated animals were bled for tests and were skin-tested at varying intervals after the tuberculous material was received.

obtained from animals which had received injections of tuberculous material. The results of the tests on these sera and the results of the tuberculin tests done at the time the sera were obtained from the animals are recorded in Table II.

All the blood sera from the animals of the nonexposed group failed to cause agglutination with sensitized pellets. Of the sera obtained from animals exposed to tubercle bacilli or to injections of phosphatide extracts of the tubercle bacillus, all exhibited positive reactions with suspensions of sensitized pellets, the height of the antibody titer varying with individual sera. Control tests with saline and normal serum gave negative results. In contrast to these results, the complement-fixation tests demonstrated the presence of complement-fixing antibodies in only 46 of the 79 sera positive to the pellet test. Skin-sensitivity was absent at the time the sera were obtained in 39 animals.

Such results suggest the use of this technic with human sera. The results of such studies are now being analyzed.

Summary. A method of utilizing suspensions of sensitized collodion pellets for the study of antibody-formation in animals during experimental studies with the tubercle bacillus is described. Suspensions of pellets sensitized with old tuberculin uniformly demonstrate the presence of antibodies against tuberculin in the blood serum of animals which have received injections of tubercle bacilli. The test clearly distinguishes sera of normal animals from those obtained from animals infected with tubercle bacilli. This method demonstrates the presence of antibodies against tuberculin in many of the animals tested at a time when the complement-fixing antibodies are not demonstrable, and at a time when many of the animals fail to exhibit skin-sensitivity to tuberculin.

11885

Isolation of Isoandrosterone from Urine in a Case of Virilism.

H. HIRSCHMANN. (Introduced by D. W. Wilson.)

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Adrenal virilism of women is frequently associated with an abnormally high titer of urinary androgens. So far two androgenic compounds, dehydroisoandrosterone and isoandrosterone, have been isolated from urine of this type. Dehydroisoandrosterone has been found in several cases of virilism (mostly tumors of the adrenal cortex),[1] but isoandrosterone has been encountered only in one instance (a case of adrenal hyperplasia)[2] and has as yet not been found in any other type of human urine. We, therefore, wish to report the isolation of isoandrosterone from the urine of a woman suffering from severe hirsutism.

S.T., a 27-year-old woman was admitted to the Urological Clinic of the Presbyterian Hospital, New York, in 1937. Examination showed a marked hirsutism of body, face, and limbs, a slightly enlarged clitoris and a hypoplastic uterus. Aerograms taken in 1937 and 1939 gave no evidence of an adrenal enlargement. On biopsy (1940) both ovaries were found to be polycystic. The clinical data do not seem to permit a diagnosis as to the gland responsible for the observed symptoms. The patient did not receive any hormonal treatment either prior to or during the period of urine collections (1937). At that time the urine contained about 100 I.U. of androgens per liter when assayed on the capon.

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[1] Crooke, A. C., and Callow, R. K., *Quart. J. Med.*, 1939, **32**, 233; Callow, R. K., *Proc. Roy. Soc. Med.*, 1938, **31**, 841; Dorfman, R. I., Wilson, H. M., and Peters, J. P., *Endocrinology*, 1940, **27**, 1.

[2] Butler, G. C., and Marrian, G. F., *J. Biol. Chem.*, 1938, **124**, 237; Marrian, G. F., and Butler, G. C., *Nature*, 1938, **142**, 400.

Urine specimens totaling 38 liters were hydrolyzed according to the procedure of Peterson, Gallagher and Koch[3] and extracted with ether within 2 weeks after their collection. The neutral ketones were obtained in the usual manner and fractionated with digitonin. The ketones precipitable with digitonin (36 mg melting 142-156°)* were acetylated. Upon recrystallization 15.2 mg of colorless needles melting at 89-94° were obtained. The final product (11.3 mg) melted at 95-96°. The melting point of isoandrosterone acetate has been given as 97° [4] and as 116°.[2, 5] It appears that this discrepancy is due to crystal polymorphism. On standing for about 18 months the acetate was partly converted into the higher melting modification (114°). A third modification of the acetate melting at 106.5° was obtained by acetylation of a synthetic preparation of isoandrosterone. While its melting point did not change on recrystallization, a mixture of all three forms was obtained on seeding with the isolated material. This preparation showed 2 points of incomplete fusion (followed by resolidification) at 97° and at 106.5° and melted completely at 116°. When the specimen was finely powdered before heating it melted sharply at 115.5-116.5°. Specimens obtained by resolidification after complete fusion usually melted at 97° and occasionally at 106.5°. A mixture of the isolated and the synthetic material melted at 113.5-115.5°.

The isolated acetate upon alkaline hydrolysis yielded colorless needles melting at 172.5-174.5°. The melting point remained unchanged after admixture with an authentic specimen of isoandrosterone melting at 174.5°. The isolated material was converted into a benzoate which melted at 217.5-219.5°. A mixture with synthetic isoandrosterone benzoate melting at 219.5° melted at 219.5°.

Examination of the mother liquors obtained in the purification of the ketones precipitable with digitonin gave no indication of the presence of dehydroisoandrosterone. This seems to be noteworthy since this compound is considered to be a normal urinary constituent.[6] The ketonic fraction which was not precipitated by digitonin yielded the 2 main 17-ketosteroids which are found in the urine of normal women,[6] androsterone and etiocholanol-3(α)-one-17.

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[3] Peterson, D. H., Gallagher, T. F., and Koch, F. C., *J. Biol. Chem.*, 1937, **119**, 185.

* All melting points reported are corrected.

[4] Ruzicka, L., Goldberg, M. W., Meyer, J., Bruengger, H., and Eichenberger, E., *Helv. Chim. Acta*, 1934, **17**, 1395; Butenandt, A., and Cobler, H., *Z. physiol. Chem.*, 1935, **234**, 218

[5] Dorfman, R. I., and Fisch, W. R., *J. Biol. Chem.*, 1940, **135**, 349.

Summary. Isoandrosterone, androsterone, and etiocholanol-3(α)-one-17 have been isolated from the urine of a woman suffering from virilism. No dehydroisoandrosterone has been found.

While this paper was in press Pearlman[7] reported the isolation of isoandrosterone from the urine of normal women and of patients with cancer. The yield which is given only for normal female urine is appreciably lower than that obtained from this case of virilism.

The author is greatly indebted to Professor G. F. Cahill, Columbia University, for permission to report his clinical observations, to Professor O. Wintersteiner, Columbia University, for his helpful advice, and to Dr. H. H. Darby, Columbia University, for the bioassay data and for arranging for the urine collections.

11886

Enhancement of Certain Toxic Effects of Codeine and Morphine by Sulfapyridine.*

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In a previous paper it has been demonstrated that sulfapyridine enhances the narcotic and toxic effects of papaverine.[1] The increase of the anaesthetic and toxic effects of barbituric compounds on rats when treated with sulfanilamide has been shown by Adriani.[2] It was considered of interest to investigate whether the action of other drugs affecting the central nervous system is also increased by sulfapyridine. The present paper deals with the influence of sulfapyridine on the effects of codeine and morphine.

The codeine experiments were performed on rats, mice, and rabbits, those of morphine only on mice. The weights of the animals ranged from 190 to 240 g for the rats, 20 to 23 g for the mice, and

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[6] Callow, N. H., and Callow, R. K., *Biochem. J.*, 1939, **33**, 931.

[7] Pearlman, W. H., *J. Biol. Chem.*, 1940, **136**, 807.

* Aided by a grant from the Sidney C. Keller Research Fund.

[1] Glaubach, S., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 325.

[2] Adriani, J., *J. Lab. and Clin. Med.*, 1939, **24**, 1066.

2000 to 2500 g for the rabbits. Sulfapyridine was administered orally to rats and mice in 5 g/kg, and to rabbits in 4 g/kg doses 15 hours prior to the subcutaneous injection of codeine or morphine. None of the animals receiving sulfapyridine alone showed central nervous system symptoms such as analgesia, catalepsy or muscle twitching.

Codeine Investigations. Rat Experiments: The type of symptoms produced by codeine is dependent upon the dosage used. Smaller doses give only sedation and analgesia which is evident by the lack of response to ear pinching. With increasing doses after the initial depression a peculiar cataleptic stage appears in which the animal can be placed first in the side, then in the sitting and back position; in this latter rigidity often occurs; the animal placed on its back remains stiff and the spine resists flexion. Competitive with these symptoms is the increase in reflex excitability due to the stimulation of the spinal cord. Large doses give rise to muscular twitching and finally convulsions. With fatal doses death occurs from respiratory failure.

Table I shows the effects of 150 mg/kg of codeine phosphate on 4 rats with and without sulfapyridine treatment. In the first ex-

TABLE I.
Effect of Subcutaneous Injection of 150 mg/kg of Codeine Phosphate into Rats With and Without Sulfapyridine.

				Time in minutes								
				Analgesia		Back position		Muscle twitching		Convulsions		
Exp. No.	Rat No.	Date	Sulfa-pyridine	O.	D.	O.	D.	O.	D.	O.	D.	Death
1	132	12/6/39	without	20	150	39	81	0	0	0	0	
2	,,	12	with	20	330	34*	296	80	10	0	0	
3	,,	22	,,	24		32		32	13	45	30	75
1	134	6	without	21	120	31	59	0	0	0	0	
2	,,	12	with	38	250	53*	267	0	0	0	0	
3	,,	22	,,	25		35*		47	13	60	50	110
1	133	6	without	20	150	39	81	0	0	0	0	
2	,,	12	,,	48	122	48	82	0	0	0	0	
3	,,	22	with	28	296	40*	270	40	70	105	7	
4	,,	1/12/40	without	45	95	33	70	0	0	0	0	
1	135	12/6/39	without	21	154	38	68	0	0	0	0	
2	,,	12	,,	23	139	38	79	0	0	0	0	
3	,,	22	with	31	314	31*	260	50	145	0	0	
4	,,	1/5/40	without	31	†	30	88	0	0	0	0	

O. Onset.
D. Duration.
*Animal becomes rigid when placed upon its back.
†Not checked.

periment the 4 rats were tested for their reaction to codeine alone. Five days later rats No. 132 and No. 134 of this group were fed with sulfapyridine, the other 2 animals remained without treatment. On the next day codeine was again administered to all 4 rats. The effects on the rats treated with sulfapyridine were enhanced in every respect, in contrast with the effects on the untreated animals. The analgesia was more persistent, the back position was maintained longer, rigidity occurred, and in No. 132 muscle twitching was observed. In the third experiment all 4 rats were treated with sulfapyridine before the codeine injection. The codeine effect was now markedly increased in rats No. 133 and No. 135 and rats No. 132 and No. 134 were thrown into convulsions and died from respiratory failure. In the fourth experiment the 2 surviving rats were tested again for their reaction to codeine alone; the effects were the same as observed in experiment 2 with codeine alone.

The experiments with 150 mg/kg codeine phosphate on another group of rats consisting of 8 animals, with 250 mg/kg on 4 rats showed results similar to those presented in Table I. After sulfapyridine the codeine effects were enhanced and delayed return to normal occurred. Of 4 animals receiving 400 mg/kg of codeine phosphate, one died. After a rest period of 6 days the remaining 3 rats received a preliminary dose of sulfapyridine before the codeine injection and all of these died.

Mice Experiments. The codeine symptoms in mice consist of an initial depression with muscular weakness and dragging of the hind legs. Elevation and bending of the tail (Straub reaction) usually occurs in this state. After toxic doses the depression is followed by restlessness, increased reflexes and convulsions; death occurs from respiratory failure. One group of 6 mice received 100 mg/kg of codeine phosphate. Straub reaction, muscular weakness and sagging

TABLE II.
Increase of Toxicity of Codeine and Morphine in Mice Treated with Sulfapyridine.

Drug	Group	No. animals	Sulfapyridine	No. of animals	
				Convulsions	Died
Codeine phosphate 200 mg/kg	A	10	with	10	10
	B	15	without	4	1
	C	12*	with	12	11
Morphine sulfate 300 mg/kg	D	15	with	15	13
	E	21	without	9	3
	F	7†	with	7	5

*The rats in this group had been used in group "B" 8 days earlier.
†The rats in this group had been used in group "E" 10 days earlier.

of the hind quarters were observed, but no convulsions occurred. The same animals treated with sulfapyridine, and then tested for their codeine reaction showed marked prolongation of the codeine effects and 2 of the animals had convulsions.

Table II shows the fatal effect of 200 mg/kg of codeine phosphate administered to 10 mice (group A) which have been fed with sulfapyridine. All animals had convulsions and died: the same doses given to 15 mice (group B) which had not received sulfapyridine produced convulsions only in 4 animals; one of which died. When 12 of these animals (group C) were fed with sulfapyridine, and then injected with codeine, all had convulsions and 11 out of 12 mice died. From this it is apparent that the toxicity of codeine is greatly increased.

Rabbit Experiments. The symptoms produced by codeine are initial depression, muscular weakness, increase in reflexes, catatonia and convulsions.

The administered doses were 20, 25 and 30 mg/kg of codeine phosphate.

The results of these experiments are as follows: 30 mg/kg of codeine phosphate administered to 3 normal animals produced toxic symptoms but no death: the same rabbits treated with sulfapyridine and then injected with codeine reacted much more strongly to the same amount of the drug, and 2 died. One rabbit which had not been tested for its reaction to codeine alone, but received sulfapyridine in the very beginning died after the injection of 30 mg/kg of codeine phosphate. Four rabbits received doses of 25, and 2 others 20 mg/kg, before and after treatment with sulfapyridine. All animals survived but their reaction to codeine was enhanced in the latter experiment. In 6 of these rabbits sulfapyridine estimations were performed immediately prior to the codeine injection. Two of the sulfapyridine rabbits which died after injection of 30 mg/kg of codeine phosphate showed blood levels of 27 and 31 mg% respectively. The values in 2 rabbits injected with 25 and 2 animals with 20 mg/kg of codeine phosphate ranged from 19-35 mg%. Six hours later when the animals appeared to have recovered completely the blood levels were still about the same.

Morphine Investigations. The effects of morphine in mice are essentially the same as those of codeine. The morphine doses used in these investigations ranged from 100 to 300 mg/kg.

For the experiments with 100 and 200 mg/kg of morphine sulfate, 12 mice were used. The animals were first tested for their reaction to morphine alone; and then after a rest period of 7 to 9 days, sulfapyridine was given preliminary to the morphine treatment. The symptoms then were more pronounced and lasted for a longer time.

The increased toxicity of morphine when preceded by sulfapyridine medication may be seen from Table II (Morphine Exp.). Fifteen mice (group D) which received 300 mg/kg of morphine sulfate after sulfapyridine, without having been tested for their reaction to morphine alone, had convulsions and 13 out of 15 died. The same amount given to 21 mice receiving no sulfapyridine resulted in only 3 fatalities. After a rest period 7 of these mice (group F) received sulfapyridine prior to the morphine injection; all had convulsions and 5 of them died.

Summary. The results reported are similar to those demonstrated for papaverine; the effect of both codeine and morphine being enhanced by sulfapyridine. The increased activity of the drugs after sulfapyridine becomes evident not only in the prolongation of their action but also in the intensity of their effects and in the increase of their toxicity.

Appreciation is expressed to Merck and Co. for supplying sulfapyridine and to Miss Bessie Zirin for her technical assistance.

11887

Attempt to Recover Sodium Pregnandiol Glucuronidate from Chimpanzee Pregnancy Urine.*

J. H. Elder. (Introduced by R. M. Yerkes.)

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Using the technic developed by Browne and Venning[1, 2, 3, 4] for the recovery of sodium pregnandiol glucuronidate from pregnancy urine, a number of investigators have shown that gradually increasing quantities of this material may be recovered throughout human pregnancy. Appreciable amounts have also been obtained during the luteal phase of the menstrual cycle.[5-8] Attempts to recover pregnandiol from pregnant rabbits, cats, and monkeys have shown negative results.[9] Marker and Hartman[10] found no trace of any of the pregnandiols in pregnancy urine of rhesus monkeys.

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*The assistance of the Committee for Research in Problems of Sex, National Research Council, is acknowledged.

The writer wishes to thank Doctor Edgar Allen of Yale University for his assistance in promoting the investigation. Considerable time was saved as a result of practical suggestions and aid from Doctors P. E. Smith, E. T. Engle, and their laboratory associates and technicians. Thanks are also due to Doctors S. R. Norris and A. F. Caraway of Jacksonville, Florida, for their generous coöperation in obtaining clinical material for the experiment.

[1] Venning, E. M. and Browne, J. S. L., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 792.

[2] Venning, E. M., Henry, J. S., and Browne, J. S. L., *Can. Med. Assn. J.*, 1937, **36**, 83, 297.

[3] Venning, E. H., *J. Biol. Chem.*, 1937, **119**, 473.

[4] Venning, E. H., *J. Biol. Chem.*, 1938, **126**, 595.

It has been found that some physiological and reproductive processes of the chimpanzee bear a closer resemblance to those of man than of monkey. Because of this fact it seemed desirable to determine whether pregnandiol could be recovered from the urine of chimpanzees. It was also hoped that in the event of positive findings an additional criterion of ovarian activity would be available. A series of tests representing various stages of pregnancy was made with two subjects. As a check on the application of the procedure, clinical specimens were extracted from time to time during the series. The procedure was exactly the same in all cases except for the method of collecting the urine specimens. With the animal subjects this was accomplished with the urine collection cage used regularly in obtaining specimens for pregnancy tests.[11] Collections

TABLE I.
Amounts of Pregnandiol Recovered from Urine of Chimpanzee and Human Subjects.

Subject	Date (1939)	Total quantity		Pregnandiol per liter (mg)	Melting point	Remarks
		cc	hr			
A	4/24	4000	24	19	255	261st day pregnancy
Soda	4/27	4500	’’	0		160th ’’ ’’
A	5/1	3750	’’	23	257	268th ’’ ’’
Soda	5/6	650	7	0		169th ’’ ’’
Alpha	5/11	800	20	0		77th ’’ ’’
’’	5/13	900	20	0		79th ’’ ’’
B	6/8	1000	8	0		25th ’’ cycle
C	8/9	3200	24	21	268	Early labor
Alpha	8/10	1800	24	0		168th day pregnancy
D	8/16	2000	24	40	271	Early labor
E	8/16	1100	24	32	263	7½ mo. pregnancy
Alpha	8/18	870	24	0		176th day ’’

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[5] Venning, E. H., and Browne, J. S. L., *Endocrinology*, 1937, **21**, 711.

[6] Stover, R. F., and Pratt, J. P., *Endocrinology*, 1939, **24**, 29.

[7] Wilson, R. B., Randall, L. M., and Osterberg, A. E., *Am. J. Obstet. and Gynecol.*, 1939, **37**, 59.

[8] Hamblen, E. C., Ashley, C., and Baptist, M., *Endocrinology*, 1939, **24**, 1.

[9] Westphal, U., and Buxton, C. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 749.

[10] Marker, R. E., and Hartman, C. G., *J. Biol. Chem.*, 1940, **133**, 529.

[11] Elder, J. H., and Bruhn, J. M., *Yale J. Biol. and Med.*, 1939, **12**, 155.

were made frequently from this cage and placed in a refrigerator until a quantity sufficient for extraction was secured. Since all specimens were used promptly after collection, only refrigeration was used for preservation.

Table I shows the results of extractions of chimpanzee urine and clinical specimens. All letters refer to the clinical cases. The practice of occasional alternation of tests with chimpanzee and clinical samples was considered necessary in order to eliminate the possibility that negative findings might be due to chance variations or inadequacies of technic. This control was indicated especially by the fact that the method was being used for the first time in these laboratories.

Pregnandiol was recovered from all of the human specimens except that from Subject B, a menstrual cycle specimen. It was never found in chimpanzee urine. The amount of final residue from the chimpanzee urines was negligible in every instance.

11888

Neutralization (*in vitro*) of Bacteriostatic Activity of Sulfonamides by P-Aminobenzoic Acid.

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Many theories have been advanced to explain the bacteriostatic activity of sulfanilamide and related compounds, but few have convincing evidence in their support. Among the more recent theories advanced is that of Fildes,[1] who proposes that inhibitors of bacterial growth are effective as a result of interference with an "essential metabolite", a substance which has an essential part in the chain of synthesis necessary for bacterial growth. The interference in the case of sulfanilamide as has been demonstrated by Woods,[2] consists in the competition for an enzyme associated with the essential metabolite and the inhibition occurs in this way because of a chemical similarity to the essential metabolite.

Woods and Fildes[2, 3] prepared a number of compounds in which the sulfonic residue of sulfanilamide was replaced by the carboxylic residue and tested for sulfanilamide neutralization. Of all the compounds of this type tested, p-aminobenzoic acid was found to be by far the most active.

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[1] Fildes, P., *Lancet*, 1940, **1**, 955.

[2] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

[3] Woods, D. D., and Fildes, P., *Chem. Ind.*, 1940, **59**, 133.

The results of our study on the effect of p-aminobenzoic acid on the bacteriostatic activity of sulfanilamide, sulfapyridine and sulfathiazole on streptococci, pneumococci, and staphylococci are reported here. In addition, the isomers and 2 derivatives of p-aminobenzoic acid have been examined for anti-sulfanilamide activity.

Anti-Sulfanilamide Activity of p-Aminobenzoic Acid. In Table I there is shown the results of a typical experiment in which p-aminobenzoic acid was examined for anti-sulfanilamide activity using *Streptococcus hemolyticus.* From this experiment it will be noted that the amount of sulfanilamide used completely inhibited the growth of streptococci in heart infusion broth. Complete neutralization of the sulfanilamide growth inhibition was brought about by the maximum concentration of p-aminobenzoic acid since the amount of growth obtained when this quantity was present approached that found in the inoculated infusion broth. Decreasing concentrations of p-aminobenzoic acid resulted in progressively diminished growth.

Anti-Sulfapyridine Activity of p-Aminobenzoic Acid. Table II gives the results of an experiment in which the ability of p-aminobenzoic acid to neutralize the bacteriostatic action of sulfapyridine on the pneumococcus was determined. As in the previous experiment, the amount of growth was in general, proportional to the concentration of the p-aminobenzoic acid.

TABLE I.

Effect of P-aminobenzoic Acid on Sulfanilamide Inhibition of *Streptococcus hemolyticus* Growth. Inoculum Streptococcus S-20, ±32.[*]

Para-aminobenzoic acid μg	Sulfanilamide μg	Growth—Colonies/1 cc in 48 hr
500	1000	690,000,000
100	”	491,000,000
50	”	440,000,000
40	”	401,000,000
30	”	310,000,000
20	”	270,000,000
10	”	145,000,000
5	”	100,000,000
1	”	373,000
.5	”	130,000
.1	”	4,000
.05	”	0
—	”	0
—	—	840,000,000

[*] *Streptococcus hemolyticus* S-20. Western Reserve University. Inoculum—1 cc 1 × 10^{-7} of a 24-hour broth culture. Medium—Difco heart infusion broth.

TABLE II.
Effect of P-aminobenzoic acid on Sulfapyridine Inhibition of *Diplococcus pneumoniæ* Growth. Inoculum Pneumococcus NY-5 ± 96.

Para-aminobenzoic acid µg	Sulfanilamide µg	Growth—Colonies/1 cc in 48 hr
100	2000	221,000,000
50	”	94,000,000
25	”	28,000,000
10	”	2,080,000
1	”	14,200
.5	”	1,680
—	”	1,000
—	—	260,000,000

**Diplococcus pneumoniæ* NY-5. Ohio State University. Inoculum 1 cc 1×10^{-7} of a 24-hour broth culture. Medium—Difco dextrose heart infusion broth.

Anti-Sulfathiazole Activity of p-Aminobenzoic Acid. The effect of the concentration of p-aminobenzoic acid on growth inhibition of staphylococcus by sulfathiazole is demonstrated in Table III. As in the 2 previous experiments the growth was proportional to the concentration of p-aminobenzoic acid, the larger the concentration of this compound, the greater the amount of growth in the presence of a constant amount of sulfathiazole.

TABLE III.
Effect of P-aminobenzoic Acid on Sulfathiazole Inhibition of *Staphylococcus aureus* Growth. Inoculum—Staphylococcus 6-14, ± 38.*

Para-aminobenzoic acid µg	Sulfathiazole µg	Growth—Colonies/1 cc in 24 hr
250	2000	440,000,000
100	”	196,000,000
50	”	85,000,000
25	”	42,000,000
10	”	36,200,000
1	”	17,200,000
0.1	”	6,080,000
	”	4,200,000
—	—	1,502,000,000

**Staphylococcus aureus* 6-14. Ohio State University. Inoculum—1 cc 1×10^{-7} of a 24-hour broth culture. Medium—Difco heart infusion broth.

Anti-sulfanilamide activity of compounds related to p-aminobenzoic acid. It was of considerable interest to determine whether the 2 isomers of p-aminobenzoic acid exhibited similar activity; accordingly o-aminobenzoic acid and m-aminobenzoic acid were tested in concentrations as high as 1 mg. No anti-sulfanilamide effect was observed. In addition, p-aminophenyl acetic acid and p-aminophenyl glycine dihydrochloride were tested in the same manner and concentration with no discernible activity.

Para-aminobenzoic acid controls were included in all of the experiments. In all cases p-aminobenzoic acid in the absence of sulfonamide inhibitor had no discernible effect on the growth of the test organisms.

Our findings on the reversal of sulfonamide inhibition of streptococcal growth by p-aminobenzoic acid are in good agreement with those of Woods. We have in addition demonstrated the *in vitro* neutralization of the bacteriostatic effect of sulfapyridine and sulfathiazole by p-aminobenzoic acid.

The data indicate that the same neutralization mechanism is apparently effective regardless of the organism or inhibitor used in these experiments. There were quantitative differences in the ability of p-aminobenzoic acid to neutralize the 3 inhibitors tested but probably not much greater than could be accounted for by the difference in the molar concentrations of the inhibitors. This suggests that the various inhibitors (sulfonamides) effect micro-organisms in a similar way even though their efficiency varies from one organism to another.

Summary. The bacteriostatic effect, as measured *in vitro,* of sulfanilamide, sulfapyridine and sulfathiazole on streptococci, pneumococci, and staphylococci respectively, has been found to be completely neutralized by p-aminobenzoic acid. This observation suggests that this neutralization mechanism is similar for any of the organisms and inhibitors investigated. The specificity of the sulfonamide neutralization by p-aminobenzoic acid is demonstrated by the inactivity of its isomers and 2 related compounds.

11889 P

Correlation Between Density and Ash, Organic, and Water Contents of Calcified Tissues.*

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There are two reliable methods for determining the density of minute particles of calcified tissues, namely (a) comparison of the refractive index with that of a liquid of known density,[1] and (b) flotation of the particle in a liquid of known density.[1, 2, 3] Knowing the density of the particle, and the relationship of density to the ash content, one then could roughly estimate the ash in particles of bone or tooth substance too small to weigh accurately. Figure 1 shows this correlation between ash and density. Table I contains data for constructing this chart and similar ones for estimation of the organic and water contents.

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* This work was supported by a grant from the Carnegie Corporation of New York.

† Now at University of Pennsylvania, School of Dentistry, Philadelphia, Pa.

[1] Manly, R. S., and Hodge, H. C., *J. Dent. Res.*, 1937, **16**, 311.

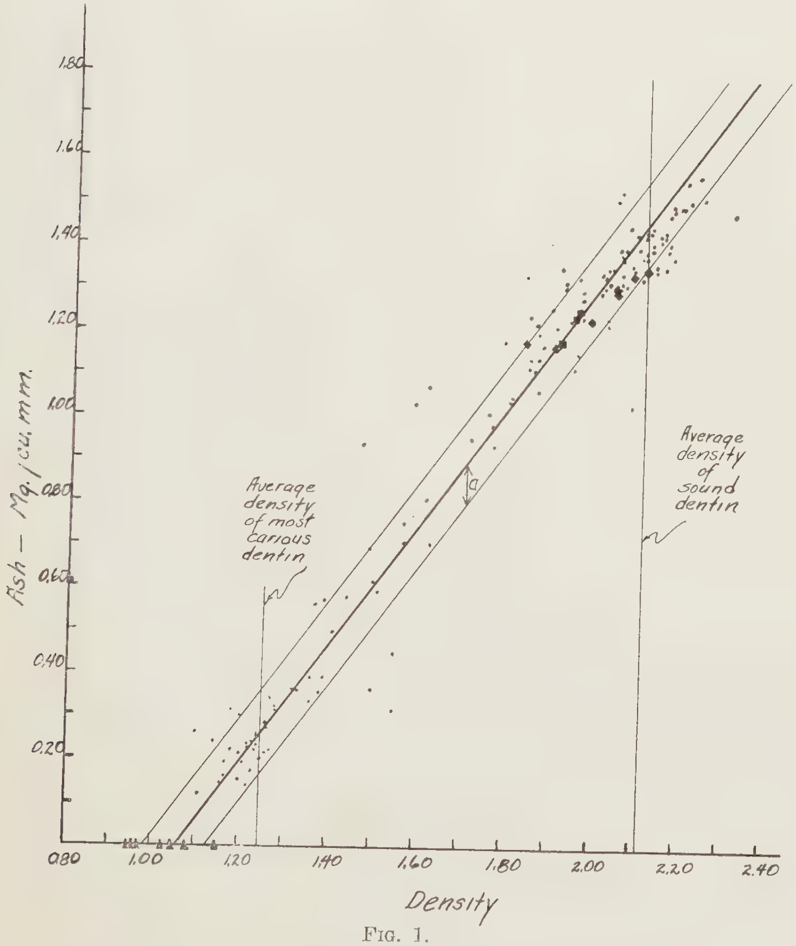

FIG. 1.

● = Dentin, normal and in varying degrees of decalcification.
▲ = Dentin, completely, artificially decalcified.
■ = Cortical beef bone.
a = One standard error of estimate.

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[2] Thewlis, J., *Brit. J. Radiol.*, 1932, **5**, 353.

[3] Brekhus, P. J., and Armstrong, W. D., *J. Dent. Res.*, 1934, **14**, 174.

TABLE I.
Data for Constructing Charts Showing Relationship Between Density and the Ash, Organic, and Water Contents of Calcified Tissues.

Function	Organic	Ash	Water
$\Sigma(y)$	67.636	142.315	55.406
$\Sigma(xy)$	121.424	282.530	85.093
a	+0.168	—1.461	+1.103
b	+0.157	+1.378	—0.427

x = abscissa (density).
y = ordinate (mg/mm³).

$$a = \text{y intercept (at } x = 0) = \frac{\Sigma(x)\cdot\Sigma(xy) - \Sigma(x^2)\cdot\Sigma(y)}{[\Sigma(x)]^2 - n\Sigma(x^2)}$$

$$b = \text{slope of line } (\operatorname{Tan}\Theta) = \frac{\Sigma(x)\cdot\Sigma(y) - n\Sigma(xy)}{[\Sigma(x)]^2 - n\Sigma(x^2)}$$

Σ = Sum.
$\Sigma(x) = 265.463$.
$\Sigma(x^2) = 486.419$.
$n = 155$.

Methods. The density of 145 samples of dentin, normal and in varying degrees of decalcification, and 10 samples of beef bone was determined while wet.[4] Ash, organic and water determinations were then made on each sample.[5] The data were treated by the method of least squares[6] and the necessary values (a,b) for construction of the charts recorded in Table I. It must be remembered that "a" is the y intercept at x=0.

11890 P

Effect of Electrically Induced Convulsions on Vago-Insulin and Sympathetico-Adrenal Systems.*

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The influence of electrically induced convulsions on the vago-insulin and sympathetico-adrenal systems was studied by determining the blood sugar of normal, adrenalectomized,† adrenalectomized-

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[4] Deakins, M. L., and Manly, R. S., *J. Dent. Res.*, 1939, **18**, 557.

[5] Manly, R. S., and Deakins, M. L., *J. Dent. Res.*, 1940, **19**, 165.

[6] Gortner, R. A., and Gortner, W. A., *J. Gen. Physiol.*, 1934, **17**, 332.

* Aided by a grant from the John and Mary R. Markle Foundation and W.P.A. Project 30278.

† This term is used for the sake of brevity. As a matter of fact the adrenal cortex was left intact.

vagotomized, and vagotomized rats. The secondary of a General Electric Variac whose primary was connected to the 110 V. 60 c.p.s. line was set at 40 volts and used as a stimulus for 0.5 and 5 seconds respectively. When the voltage was applied for 5 seconds it was timed with a clock, in the case of the 0.5 second application an automatic relay switch was used. Two paper clips clamped to the shaved skin over the skull served as electrodes. Blood samples were taken before and at various intervals after the convulsions. The stimulus produced regular convulsions in each instance in which first a flexor phase predominated which was then followed by tonic extension of the extremities. During the application of the stimulus urination usually occurred. The righting reflex returned by the end of the second minute but even after that time a state of catalepsy was observed for several minutes. Table I shows the effects of these convulsions on the blood sugar in the 4 groups of animals. Vagotomized and normal animals show a hyperglycemic response which is greater in the former. This seems to indicate that both vago-insulin and sympathetico-adrenal systems are stimulated during convulsions, an effect which is similar to that of metrazol.[1] Moreover, the experiments indicate that the effect on the sympathetico-adrenal system predominates over that of the vago-insulin system. These conclusions are confirmed by the fact that the elimination of the sympathetico-adrenal system by demedullation of the adrenals makes the effects on the vago-insulin system manifest as evidenced by the hypoglycemia resulting from the convulsions in the adrenalectomized animals. If the interpretation is correct that the adrenal medulla and the Islands of Langerhans are stimulated only via the sympathetics and the vagus respectively, no significant changes in blood sugar are expected in animals in which the vagi were cut below the diaphragm and the adrenals demedullated. The table shows that this is indeed the case.

TABLE I.
Effect of Convulsions Produced by Electric Shock of 0.5 sec. Duration on the Blood Sugar of Rats.

	Before convulsions	Blood sugar in mg% (avg of 6 animals) After convulsions		
		10 min.	40 min.	70 min.
Controls	74.3	88.4	101.1	82.4
Adrenalectomized	63.4	55.5	43.5	64.4
Adrenalectomized-Vagotomized	66.0	67.9	65.0	66.4
Vagotomized	83.8	100.1	110.5	102.6

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[1] Feldman, J., Cortell, R., and Gellhorn, E., *Am. J. Physiol.*, 1940, **131**, 281.

Summary. Electrically induced convulsions produce a hyperglycemia in the normal, a hypoglycemia in the adrenalectomized rat, and no change in blood sugar in adrenalectomized-vagotomized animals. This is interpreted to mean that the convulsions excite both the sympathetico-adrenal and vago-insulin systems. The effect on the former predominates over that on the latter. If a normal animal the vagi are cut below the diaphragm and therefore the secretion of insulin can no longer be controlled by the vagus, it is found that the hyperglycemic response is greater than in control animals in which the vagi are intact.

11891

Blood Volumes in Hypertensive Partially Nephrectomized Rats.*

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From the Biochemical Laboratory, University of Virginia, University, Virginia.

Most clinical investigators have found no parallelism between hypertension and blood volume.[1] Dogs with experimental renal hypertension produced by the Goldblatt procedure[2] have normal cardiac output[3] and normal blood volume.[4, 5] Griffith and Ingle[6] believe that they have demonstrated an increased blood volume in hypertensive partially nephrectomized rats.

In the present study it is shown that the blood volume is not a factor in the maintenance of an increased blood pressure in partially nephrectomized rats.

Methods. Male rats of Wistar strain, subjected to partial nephrectomy,[7] after a period of 3 to 4 months following operation, were

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* Supported by a grant from the John and Mary R. Markle Foundation.

† Nemours Foundation Fellow.

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[2] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[3] Freeman, N. E., and Page, I. H., *Am. Heart J.*, 1937, **14**, 405.

[4] Holman, D. B., and Page, I. H., *Am. Heart J.*, 1938, **16**, 321.

[5] Gibson, J. G., and Robinson, R. W., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 497.

[6] Griffith, J. Q., and Ingle, D. W., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 538.

[7] Chanutin, A., and Ferris, E. B., Jr., *Arch. Int. Med.*, 1932, **49**, 767.

used as experimental animals. Only those partially nephrectomized rats showing the presence of hypertension, as judged by the tortuosity and dilatation of the carotids, were studied. Intact animals of the same age were used as controls.

After anesthetization with an intraperitoneal injection of sodium pentobarbital, blood volumes were determined by a modification of the dye method of Gibson and Evans[8] and Gibson and Evelyn.[9] A 1% aqueous solution of Evans blue (T-1824) was injected into the jugular vein through a hypodermic needle. The amount of dye injected varied between 0.2 and 0.3 cc depending on the weight of the animal. The dye was measured in a serological pipette connected to the needle by means of a rubber tube and a 3-way metal stopcock. After 10, 20, 30, and 40 minutes following the injection of dye, approximately 0.2 cc of blood was collected from the cut tail at each sampling in a capillary pipette. After the blood clotted, the pipette was sealed and centrifuged and .02 cc of the dyed serum was diluted with 1.5 cc of distilled water. The photometric densities were determined with the micro attachments of the Evelyn photoelectric colorimeter and the results were extrapolated as described by Gibson and Evans.[8] Distilled water was used as the blank for the colorimeter since there was no difference in the center setting after diluting the serum 75 times, even when hemolysis was present. A drop of blood was collected in a machine-drawn capillary tube and the relative concentrations of corpuscles and plasma were measured. In order to prevent clotting, a weak solution of heparin was drawn through the tubes which were then dried.

The following formulae[9] were used for calculating the total and plasma blood volumes:

$$\text{P.V.} = \frac{\text{M K} \times 100}{\text{L} \times 75}$$

and

$$\text{B.V.} = \frac{\text{P.V.}}{\text{100-Hematocrit}}$$

where P.V. = plasma volume, M = mg of dye injected, K = constant, L = photometric density, 75 = dilution factor, and B.V. = blood volume.

Results. The individual data for total blood volume, plasma and red cell volumes in the intact and partially nephrectomized rats are shown in Fig. 1.

Intact Rats. The average data and their standard deviations for

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[8] Gibson, J. G., 2nd, and Evans, Wm. A., Jr., *J. Clin. Invest.*, 1937, **16**, 301.

[9] Gibson, J. G., 2nd, and Evelyn, K. A., *J. Clin. Invest.*, 1938, **17**, 153.

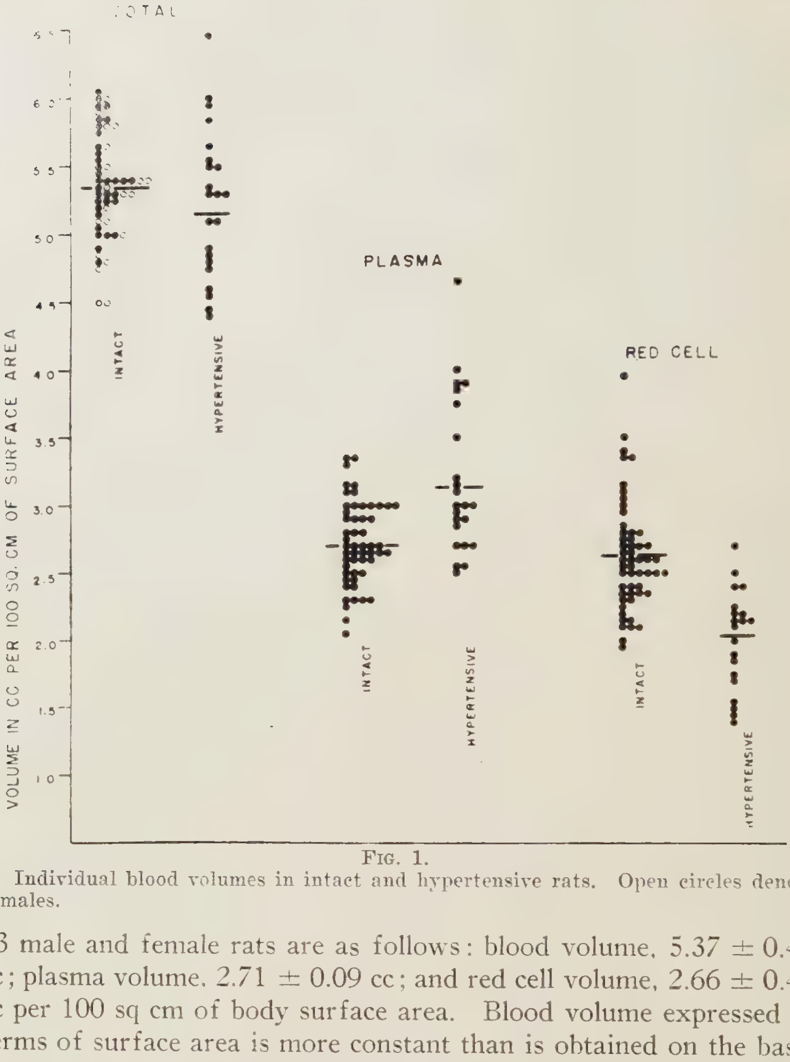

Fig. 1.

Individual blood volumes in intact and hypertensive rats. Open circles denote females.

53 male and female rats are as follows: blood volume, 5.37 ± 0.41 cc; plasma volume, 2.71 ± 0.09 cc; and red cell volume, 2.66 ± 0.41 cc per 100 sq cm of body surface area. Blood volume expressed in terms of surface area is more constant than is obtained on the basis of body weight and is not affected by the size or sex of the rat. The average values for these same rats expressed as cc per 100 g of body weight are as follows: blood volume, 7.98 ± 0.85; plasma volume, 4.08 ± 0.49; and red cell volume, 3.90 ± 0.73.

The average value for 20 intact female rats was 5.32 cc per 100 sq cm of body surface as compared with a value of 5.43 cc for male animals. The individual distribution for males and females is shown in Chart 1.

Hypertensive Rats. The average data and their standard deviations for 21 partially nephrectomized rats are as follows: blood volume, 5.16 ± 0.55 cc: plasma volume, 3.13 ± 0.57; and red cell volume, 2.03 ± 0.41 cc per 100 sq cm of surface area. A comparison of these values with those of the intact rats shows no significant difference for the total blood volumes. There is, however, an appreciable increase in the plasma volume and a corresponding decrease in the red cell volume, due to the anemia developed in these animals.[7]

Comment. The results obtained in this work for intact rats are similar to those reported by Went and Drinker.[10] These workers, using a micro-dye method obtained an average total blood volume of 7.4 cc per 100 g of body weight and 5.1 cc per 100 sq cm of surface area.

Griffith and Ingle[6] recently reported that hypertensive partially nephrectomized rats showed an increase in total blood volume, which would appear to be contrary to the findings reported in this paper. On inspection of their procedure for estimating blood volume,[11] it appears that they neglected to take the red cell volume into consideration and the values reported by them probably represent plasma volumes. In the present experiments, the increased plasma volumes in partially nephrectomized rats agree with the reinterpreted data of Griffith and Ingle.

Summary. Plasma and total blood volumes were determined in 33 normal male and 20 normal female rats. In the hypertensive partially nephrectomized rat, the plasma volume was increased, the red cell volume decreased and the total blood volume remained unchanged. It is concluded that hypertension is not associated with increased blood volumes in these experiments.

11892

Relationsip of Moscow 2 Virus of Equine Encephalomyelitis to Rabies.

Beatrice Howitt.

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The virus designated as the Moscow 2 strain by Vichelessky and coworkers[1] was placed by them in the group of neurotropic agents causing encephalomyelitis in horses. The Russian workers had recognized a serological relationship to the virus of rabies but were unable to demonstrate clearly defined Negri bodies in the tissue cells of the animals examined.

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[10] Went, Stephan, and Drinker, C. K., *Am. J. Physiol.*, 1929, **88**, 468.

[11] Griffith, J. Q., and Campbell, R., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 38.

[1] Vichelessky, R. S., Naskov, A., Soukhof, M., and Moutovine, V., *Rec. med. vet. l'École d'Alfort*, 1935, **3**, 357.

In order to determine the extent of this relationship, further comparisons have been made with a known fixed rabies virus.* The earlier observations were confirmed by these studies and several animal experiments have been made in addition.

Anti-rabic immune serum was mixed with an equal amount of the Moscow 2 virus, diluted 1:5,000 and 1:10,000 respectively, placed in the refrigerator over night and inoculated intracerebrally into white mice the next day. Neutralization occurred on each of several occasions, while the control mice given mixtures of virus diluted in normal serum and broth, succumbed to the disease. In like manner, complete protection occurred when the Moscow 2 hyperimmune rabbit serum was tested against the known fixed rabies virus in dilutions of 1:5,000 and 1:10,000 respectively. These results could also be repeated.

A guinea pig hyperimmunized against the Russian strain was found to be immune to an intracerebral inoculation of undiluted rabies virus which killed the two control animals in 4 days. Four white mice immune to the rabies virus failed to succumb to intracerebral injection of the undiluted Moscow 2 virus which killed 3 control animals in 5 to 6 days.

Serum from rabbits immunized with suspensions of live rabies virus isolated from a dog in San Francisco gave strong reciprocal reactions with the Moscow 2 strain. The former virus had been recovered from an animal showing an atypical clinical appearance, a slow paralytic form of disease rather than the acute, furious type. Reciprocal reactions occurred, however, with both the immune serums and viruses, thus establishing the identity of the two viruses. In the same manner, the Moscow 2 antiserum protected against another strain of rabies isolated from a duck and against yet another somewhat atypical one. The latter was recovered under rather unusual circumstances and did not produce intracytoplasmic inclusions in the brain cells of any animal tested except the dog.

Previously no reciprocal reactions of any kind had been demonstrated for this Russian strain. In a former report[2] it had been shown that the Moscow 2 virus was serologically different from both

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* Both the rabies virus and the anti-rabic immune serum first used were kindly sent by Dr. Anson Hoyt of the University of Southern California.

[2] Howitt, B. F., *J. Inf. Dis.*, 1938, **63**, 269.

American strains of equine encephalomyelitic virus, about the only point in common being the recovery from the horse. There was no cross tissue immunity with either the eastern or the western equine strains, nor any cross neutralization or complement fixation with these or with the viruses of lymphocytic choriomeningitis, Japanese B and St. Louis encephalitis, respectively. It differed from the 2 viruses of equine encephalomyelitis in its larger size, its ability to induce a lower fever reaction in the guinea pig, its inability to be readily cultivated on the membranes of the developing chick, its absence in the blood stream and visceral organs and the nontransmission of immunity to the young guinea pig through the placenta. It did, however, have the same 4-day incubation period in the guinea pig characteristic for the western equine strain and often induced similar clinical symptoms, although occasionally animals showed a difference by manifesting extreme spasticity and a convulsive state. In mice the incubation was longer than for the western virus. White rats, the cotton rat *(Sigmodon hispidus eremicus)*, cats, mice, rabbits and monkeys were found to be susceptible in the same manner as they were for the 2 equine strains. A 6-pound duck and two 3-pound chickens failed to respond to intracerebral inoculation after 7 weeks' observation. Three chicks of one week and 2 of 2 weeks' age, hatched in the laboratory, however, became prostrated or died in 2 to 3 weeks, respectively, after intracerebral injection of undiluted virus. The virus was recovered from the brain of the chicks.

The Russian workers recognized the resemblance of the Moscow 2 strain to rabies virus, yet they placed it in the equine group of neurotropic viruses largely because of failure to infect dogs or to find Negri bodies in the tissues examined. In order to make further observations in this regard, two 6-weeks-old puppies were inoculated with the undiluted Moscow 2 virus, one with 0.4 cc intracerebrally and the other with 2 cc subcutaneously. The former animal was sacrificed after complete prostration in 5 days, the virus being recovered from the brain and cord. The other dog became very weak in the hindlegs, although with some spastic reactions, then developed a spastic paralysis of the forelegs, combined with a generalized convulsive twitching, and finally became prostrate on the 23rd day after the injection. The virus was demonstrated in the brain and cord after autopsy.

Vichelesky and coworkers[1] did not report the presence of Negri bodies within the nerve cells for the Moscow 2 strain, yet, because of the short incubation period for most animals, it is likely that the inclusion bodies were not sufficiently developed to be readily seen. It is known that these inclusions are not always demonstrable in tissues from rabid animals, especially in the acute type of the disease. That rabies may appear in several forms, both unusually acute as well as with a prolonged course, has been recognized for some time. The virus reported by Koritschoner[3] and that by Jonnesco[4] both manifested extremely short incubation periods and failed to produce Negri bodies in small laboratory animals. It was not until repeated passage in the dog that they were found with the latter strain. Later Pawan[5] recovered a rabies virus having a 2-day incubation period from rabbits bitten by a vampire bat. No Negri bodies were demonstrated until after the incubation was prolonged by intrasciatic inoculation of rabbits with the virus.

As previously reported,[2] small intracytoplasmic bodies were found by Covell in the brain cells of a cat and a monkey given the Moscow 2 strain, but not in those of the guinea pig and the rabbit. They were again demonstrated in cells from the brain of the dog given the subcutaneous injection.

To determine if more typical inclusion bodies could be produced, the Russian virus was inoculated into the sciatic nerve of a monkey. After 9 days it developed violent convulsions, frothing at the mouth and the general appearance of a rabid animal. It was sacrificed and examination revealed the presence of the virus in the brain and cord at different levels after passage to white mice. Medium sized, round acidophilic bodies were found by Covell in the cytoplasm of the brain cells when stained with rosaniline methylene blue by the Schleifstein method.[6] The inclusions were different from the usual Negri bodies, however, in that no basophilic granules were noticed, but appeared more like the bodies described for rabies by Manouelian.[7] The inclusions demonstrated in the tissue cells of the other animals examined, dog, cat, and monkey, were all of this general type, rather small and compact and not of the appearance usually seen in the cells of dogs infected with the common street virus. Since it is known that typical Negri bodies are not usually observed with fixed virus material and since the Moscow 2 strain has been passed for a number of years, showing a definite incubation period it is not unlikely that it has become "fixed" for laboratory animals and thus the original form of inclusion has been modified.

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[3] Koritschoner, *Wien. klin. Wschr.*, 1923, **36**, 385.

[4] Jonnesco, D., *Ann. l'Instit. Past.*, 1932, **49**, 435.

[5] Pawan, J. L., *Ann. Trop. Med. and Parasitol.*, 1938, **32**, 35.

[6] Schleifstein, J., *Am. J. Pub. Health*, 1937, **27**, 1283.

[7] Manouelian, Y., *Ann. l'Instit. Past.*, 1914, **28**, 233.

References may be found in the literature to various atypical forms of the rabies virus, yet Remlinger and Bailly[8] have not recognized any so-called "plurality of viruses". They rather prefer to account for the differences among strains as due to the differences in activity and invasiveness, especially if a serological and immunological relationship has been demonstrated. That the infectiousness may vary considerably has been illustrated by the reports previously mentioned of Koritschoner,[3] Jonnesco,[4] and Pawan.[5] In these instances the short incubation periods and the failure to demonstrate Negri bodies readily were disturbing factors, remedied only after the serological and immunological experiments had shown a definite relationship to rabies virus. For this reason Remlinger and Bailly consider it misleading to rely solely on the finding of Negri bodies, because of the occasional acceleration of the incubation time with coincident failure to produce intracytoplasmic inclusions in many animals. They would depend largely upon the serological and immunological tests to establish the relationship of an unknown strain to that of rabies as was done for the viruses mentioned above.

While the relationship of the Moscow 2 strain to that of rabies has been demonstrated by the serological, immunological and cytological experiments, the particle size, 80 to 130 millimicrons,[9] would also place this virus closer to rabies than to the American strains of equine encephalomyelitis. The range of the latter lies between 20 to 30 millimicrons,[2] while that of rabies is placed by Galloway and Elford[10] at 100 to 150 millicrons for fixed rabies virus; Levaditi and coworkers[11] have found higher figures, 140 to 210 millicrons for the fixed virus and 160 to 240 millicrons for the street virus.

Summary. From the serological and immunological reactions, from the difficulty in filtration, from the particle size, from the absence of virus in the blood and organs, from the ability to infect dogs both intracerebrally and subcutaneously, and from the presence of intracytoplasmic inclusions, although slightly atypical and not inclusive for all animals, the Moscow 2 virus may now be classified with the rabies strains rather than with the American viruses of equine encephalomyelitis as has formerly been suggested.

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[8] Remlinger, P., and Bailly, J., *Bull. Inst. Past.*, 1931, **29**, 1.

[9] Lazarus, A., and Howitt, B., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 595.

[10] Galloway, I., and Elford, W. J., *J. Hyg.*, 1936, **36**, 532.

[11] Levaditi, C., Paic, M., and Krassnoff, D., *C. R. Soc. Biol.*, 1936, **123**, 866.

11893 P

Acute Edema of the Pancreas, Gallbladder and Stomach in Serum Anaphylaxis.

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As far as we are aware, the occurrence of acute edema of the gallbladder, pancreas and stomach has not been described as a consequence of serum anaphylaxis in the dog.

Incidence. In 21 dogs, we observed edema of the pancreas 8 times; edema of the gallbladder-mucosa and adventitia 15 times: and edema of the stomach-submucosa 8 times.

Degree of Edema. This varied considerably; in the *pancreas* the entire gland could be involved so that the lobules were separated by a clear, watery fluid; or the edema was largely limited to that portion closely adherent to the duodenum and antrum.

The lymph glands between the pancreas and stomach were generally black, hard and gorged with blood. Other lymph glands of the viscera appeared normal.

In the gallbladder the edema of the mucosa and adventitia varied between 1 and 3 mm in thickness; the fundus was chiefly involved and the edema was less on the body and apparently absent on the neck of the bladder.

In the stomach, the edema was found chiefly in the submucosa of the antrum and especially of the preantrum, and was most marked along the lesser curvature; the fundus generally showed no edema. In several instances, however, the fundus showed limited areas of edema at least 5 mm in thickness and this edema was associated with definite thickening of the mucosal rugae.

The duodenal submucosa never showed any appreciable edema.

The edema fluid in all instances was water-clear and not bloody.

A few petechial hemorrhages into the gastric mucosa of the fundus were observed in only one dog.

Duration. Some evidence is available indicating that the edema becomes less and disappears as the animal recovers.

It is conceivable that this acute edema of the gallbladder, pancreas and stomach may serve as the starting point of pathological processes or exacerbate existing abnormal conditions in the involved organs.

The dogs weighed approximately 10 kg and were sensitized by either one or several parenteral injections, each of 5 cc horse serum.

The incubation period varied between 21 and 38 days. The reinjection dose was always 20 cc normal horse serum, intravenously. Blood pressure, coagulation time, hemoglobin, etc., were recorded throughout. Ether was discontinued after the blood pressure had reached a low level Biopsy was performed generally within 30 minutes after the shocking dose of serum.

11894 P

Acute Edema of the Pancreas, Gallbladder and Stomach After Histamine Injections.

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Since acute serum anaphylaxis produced well marked edema of some abdominal organs in the dog,[1] it became desirable to test the effect of histamine in this direction, especially as a search of the literature gave no information on this question.

Incidence. In 14 dogs, edema of the pancreas occurred 6 times; edema of the gallbladder 14 times; of the stomach 7 times.

Degree of Edema. This varied as has been described in serum anaphylaxis;[1] on the whole, it seemed not as strong as in the serum dogs, yet it must be noted that occasionally degrees of edema were seen in the pancreas, gallbladder and stomach that were equal to those observed in serum anaphylaxis.

The *location of edema* was the same as in serum anaphylaxis. The edema fluid in all instances was clear and not bloody.

The *lymph glands* between the pancreas and duodenum were brown red in color, and showed red areas on section; they were never black and hard as in serum anaphylaxis.

The dogs were males and weighed between 6 and 8 kg; the histamine hydrochloride was infused into a jugular vein in a 0.1% solution using a Mariotte burette; the dose ranged between 2 and 6 mg per kg. Biopsy was performed within 30 minutes after the fall of blood pressure. Ether was discontinued as soon as the blood pressure had reached a low level.

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[1] See preceding article.

11895

The 17-Monocaprylic Ester of Estradiol—A Highly Active Tumorigenic Estrogen.*

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The 17-caprylate of α-estradiol has been shown to be more tumorigenic than the monobenzoate and dipropionate esters.[1, 2] This finding has led us to examine quantitatively how greatly the tumorigenic action of estradiol is enhanced when it is esterified with caprylic acid. When 200 γ of free estradiol are injected thrice weekly in the course of 3 months into a castrate female guinea pig, there is little or no fibrous reaction: 400 γ per injection, or a total of 16 to 20 mg of free estradiol, are necessary to induce in a 3-month period a definite tumoral reaction.[3] We therefore investigated the amount of the caprylic acid ester necessary to cause a similar reaction.

When 1 γ of estradiol given as the monobenzoate or dipropionate ester[†] was injected thrice weekly (a total of 37 to 38 γ in the course of 3 months) no fibroids, tumoral seed on the spleen, or fibrous strands in the mesentery were visible to the naked eye. Microscopical investigation revealed minute mesometrial fibroids in one out of 10 animals (Fig. 1).

Of 17 animals which received the caprylic acid ester, uterine tumors visible to the naked eye were found in 2 which received during a 3-month period 18 and 41 injections of 1 γ respectively. No stress can be laid as yet on these 2 findings since the microscopical structure of these 2 tumors was unlike the experimental fibroid in

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* This investigation has been aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research, and Mr. Adolfo Eastman of Limache (Chile). The 17-caprylic ester was generously supplied by Dr. Carl Miescher of Ciba Pharmaceutical Products, Inc.

[1] Jedlicky, A., Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol* (Paris), 1939, **130**, 1466.

[2] Lipschütz, A., Bellolio, P., Chaume, J., and Vargas, L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 164.

[3] Lipschütz, A., Rodríguez, F., and Vargas, L., Jr., *C. R. Soc. Biol.* (Paris), 1939, **130**, 939.

[†] 1 γ of estradiol is contained in 1.38 γ of the monobenzoate, in 1.41 γ of the dipropionate and in 1.46 γ of the monocaprylate.

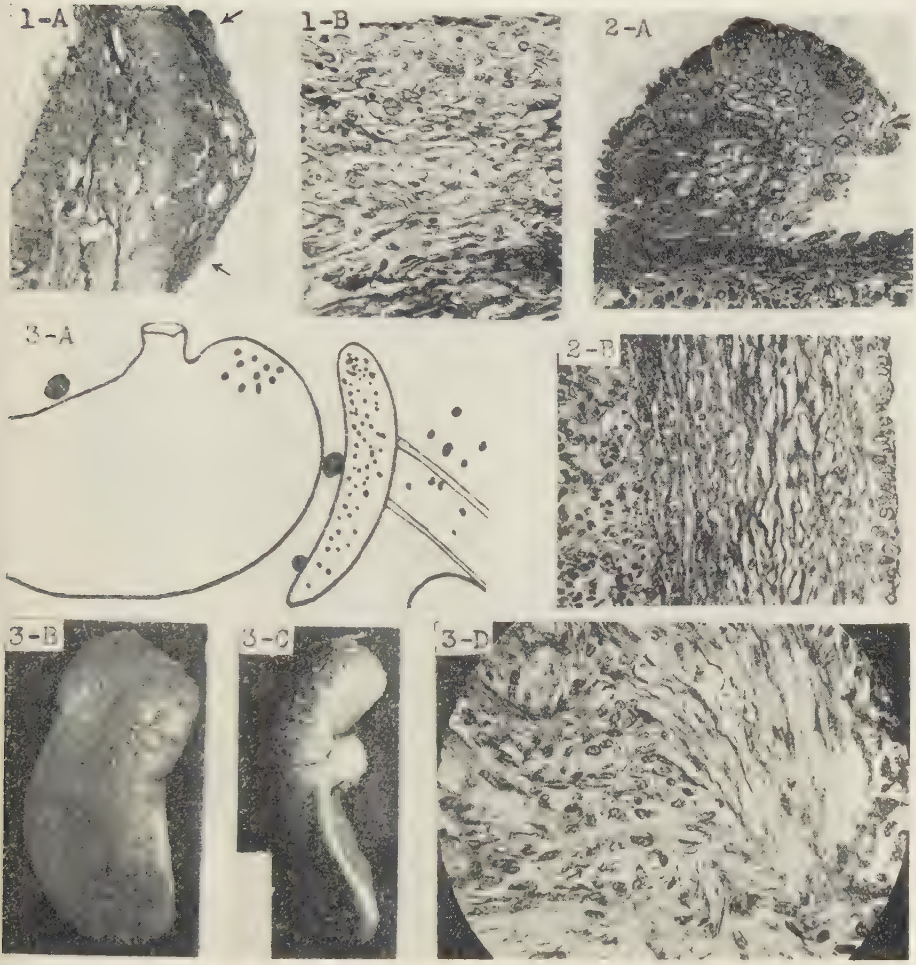

FIG. 1. Two minute fibroids near the mesometrium, discovered only at microscopical examination. Animal received 37 injections of 1 γ of estradiol (monobenzoate) in the course of 85 days. A. × 10. Arrows indicate fibroids. B. × 200. Peripheral part of minute fibroid. Note spindle shaped cells (fibroblasts?) and flattened cells on the surface. Fibrous tissue nearer to the center (VI, 5).

FIG. 2. A. × 200. Small excrescence on the spleen produced by 10 injections of 1 γ of estradiol (in form of the 17-caprylate) in the course of 21 days. Cells rich in protoplasm at the periphery and sclerotic tissue nearer to the center. Capsule of the spleen thickened. Cells of peritoneal mesothelium increased. B. × 200. Same animal. Fibrous patch of greater extension on the spleen. Cells rich in protoplasm at the periphery, and below them, spindle shaped cells separated from one another by thick fibers (XVII, 12-B).

FIG. 3. A. Diagram to show localization and size of fibroids produced by 38 injections of 2 γ of estradiol (in form of the 17-caprylate) in the course of 88 days. Splenic and gastric fibroids. Tumoral seed on spleen, stomach and abdominal wall. B and C. × 1.4. Dorsal view of the spleen of the same animal, with tumoral seed and tumor in the hilum (XXI, 13). D. × 200. Fibroid in the hilum of the spleen. Abundance of cells, fibrous strands and probably also smooth muscle fibers (XXI, 11).

TABLE I.
Ten Castrate Female Guinea Pigs Treated with Subcutaneous Injections of the 17-caprylate of Estradiol During 3 Months.

Quantity* of estradiol inj. thrice weekly γ	No. of inj.	Total No. animals	No. of animals with some kind of conjunctive reaction	No. of animals with tumors size class 1 or over	Avg "total tumoral effect"
2	38	5	4	2	2.3
3	38	5	5	5	2.5

*The weight of estradiol is given. 1γ of estradiol is contained in 1.46 γ of the caprylic acid ester.

general. Tumoral seed on the spleen (Fig. 2) and other parts of the abdominal cavity was found after use of the caprylate in 10 out of 46 animals which received 10 to 40 injections of 1 γ in the course of 21 to 93 days. One may indeed question how far the tumoral seed is to be identified with experimental fibroids in general.

The results are very different when 2 to 3 γ of the caprylic ester per injection were given (Table I). Not only a tumoral seed on the spleen but also splenic and other fibroids were produced (Fig. 3) with a total amount equivalent to only 76 γ of estradiol and injected in the 3-month period. With this minute quantity of estradiol administered as the 17-caprylate the tumoral reaction is much more considerable than with 5 or even 10 γ of the monobenzoate or dipropionate per injection.[2] The tumorigenic action of 2 γ of the caprylate is also enormously superior to that of 200 γ of free estradiol.[3, 4] *Under the given timing conditions (3 injections weekly) the tumorigenic action of free estradiol is enhanced by esterification with caprylic acid from one to two hundred times.* On the contrary, the hysterotrophic or "physiological" dose[3] of free estradiol is certainly no more than 10 times that of the 17-caprylate. This gives full evidence that esterification, especially with caprylic acid, confers upon estradiol under certain timing conditions greater tumorigenic potency. When roughly expressed in figures the tumorigenic quotient, or $Q_{tum} = \frac{\text{tumorigenic dose}}{\text{hysterotrophic dose}}$, is for the 17-caprylate less than 20 ($Q_{tum} = <2/0.1$), and for free estradiol more than 200 ($Q_{tum} = >200/<1$).

Summary. The conjunctive tumorigenic action of α-estradiol is so greatly enhanced by esterification with caprylic acid at position 17 that under certain timing conditions it becomes 100 to 200 times greater than that of free α-estradiol. With 38 injections of the 17-caprylate of estradiol given in the course of 3 months and equivalent to a total of 76 γ of estradiol, typical experimental abdominal fibroids can be induced.

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[4] Rodríguez, F., *Public Med. Exp.* (Chile), 1940.

11896

Reaction to Chilling as an Index of Tolerance for Ingested Sulfonamide, in the Rabbit.

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Massive doses of sulfanilamide produce a depression in ability to recover body temperature after chilling,[1] in the rabbit, equivalent to that produced by morphine in toxic dosage.[2] The effect is examined, in this report, for relation to quantity fed, blood level reached, percent weight loss induced and percent fatality. Comparative examination is made of sulfapyridine and sulfathiazole.

The ability to recover body temperature after chilling is appraised from observation of the number of minutes required to accomplish a rise from 96 to 99°F after chilling to a point between 95 and 96°F as described in reference 2. The number of minutes so measured is reported as the warming time. Warming times between 28 and 40 are within the normal range. Serious impairment in capacity for temperature recovery after chilling is indicated by values substantially longer than 45.[2]

TABLE I.
Effect of Ingested Sulfonamide on Warming Time as Related to Amount Fed, Blood Level Reached, Per Cent Weight Loss and Mortality.

Drug	Amt given g/kg	Blood level at time of test, mg %*		No. of rabbits	% with a warming time lengthening of		% with total wt losses of >5% or per diem losses of >3%	% deaths
		range	avg		>16%	>60%		
SA	.10-.15ad	4- 8	5	6	33	0	17	0
	.26-.36de	12-40	20	7	100	57	100	14
SP	.14-.29ad	1- 7	4	4	25	0	25	0
	.30-.55abd	2-27	11	8	75	25	75	13
ST	.15-.36adf	3-16	7	10	10	0	0	0
	.48-.72de	4-32	15	7	72	29	43	14
none†				295	14	0.3	5	0

Abbreviations: SA, sulfanilamide; SP, sulfapyridine; ST, sulfathiazole.

a: given once only. b, c, d, e, f: given 3 times daily for 1, 2, 3, 4 and 8 days, respectively. The dosage was progressively increased from rabbit to rabbit, in each group, but was held at a constant level for each individual rabbit compared.

*Free, non-conjugated substance.

†Rabbits comparably observed, over a period of 3 to 8 days, for range of spontaneous change in warming time and weight.

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[1] Locke, A., and Main, E. R., *J. Immunol.*, 1939, **36**, 173.

[2] Locke, A., *J. Infect. Dis.*, 1937, **60**, 106; *J. Immunol.*, 1939, **36**, 159

Table I compares the effect of sulfanilamide (SA), sulfapyridine (SP) and sulfathiazole (ST) on the warming time following feedings in the indicated amounts over the indicated number of days. The rabbits used were New Zealand White males averaging 3 kg in weight. They had not been losing weight before the experiment was begun and had normal rectal temperatures. The average warming time before the drug feedings was 35. The drugs were given in tablet form with water.* The blood level estimations[3] were made on samples drawn from the marginal vein of the ear 2 to 3 hours after the final drug feeding and immediately before the test for degree of warming time lengthening induced.

The lengthenings observed did not persist following discontinuance of the drug feedings.

Summary. Sulfanilamide, sulfapyridine and sulfathiazole produced a lengthening of the warming time, in the rabbit, roughly proportional to the amount fed and the blood level reached. Serious (>60%) lengthening occurred to an extent paralleling the incidence of rapid weight loss and death.

11897

Stability of Prothrombin in the Presence of Thrombin.

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In earlier numbers of these PROCEEDINGS, Mertz, Seegers and Smith[1, 2] allege that thrombin can inactivate its precursor prothrombin. We have published evidence[3] that a trypsin-like enzyme is not only a thromboplastic agent[4] but also a *progressive* antithrombin[5] of major importance in natural thrombin destruction. It is present, to a varying extent, in serum and in most prothrombin and thrombin preparations. There is doubt whether such a factor was ruled out in the Iowa experiments. It should be pointed out that Smith, *et al.*, use extremely potent thrombic agents and assay them by a dilution technic which yields values up to several thousands. In our experience, strong thrombins (and prothrombins) *appear* unusually stable but turn out to be much less so when diluted to, say, about 5-25 Iowa units. In Table I of another Iowa communication,[6] there is a 9% loss of activity of thrombin in the presence of prothrombin as compared with a much more startling 95% fall in the prothrombin titer during the same 4-hour period.

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* The tablets were pushed through the side of the mouth, and as far back as possible, with the right forefinger. The mouth was kept moist and the finger held against the disintegrating material until swallowing was complete.

[3] Bratton, C. A., and Marshall, E. K., *J. Biol. Chem.*, 1939, **128**, 537.

[1] Mertz, E. T., Seegers, W. H., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 657.

[2] Mertz, E. T., Seegers, W. H., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 604.

[3] Ferguson, J. H., and Erickson, B. Nims, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 625.

[4] Ferguson, J. H., and Erickson, B. Nims, *Am. J. Physiol.*, 1939, **126**, 661.

Reagents and Methods. Our routine prothrombins,[7] when maximally activated with $CaCl_2$ and brain thromboplastin, usually clot a test fibrinogen in some 5-20+ seconds (temp. = 37.5°C, pH = 7.5). A typical prothrombin solution, assayed through the courtesy of Dr. H. P. Smith (Iowa) gave 47 units per cc (38 units per mg N). Persisting in our unwillingness to use any system of thrombin "units" for comparing one preparation with another, without convincing proof of control of *instability factors,* we restrict the assay to a single prothrombin preparation and the thrombins freshly prepared from it. Under these narrowed conditions, thrombin concentrations are measured, with a 5-10% accuracy, in a relative manner from the clotting-times obtained with a series of dilutions tested under strictly comparable conditions. In favorable cases, there is close approximation to the "inverse law,"[5] *viz.,* thrombin concentration varies inversely as the clotting-time. Since there is often difficulty in establishing the simple linear relation, it is more practicable to read the thrombin strengths directly off a *dilution curve* plotted graphically, as in Fig. 1 (Curve 0).

In the current experiments, typical of several such series, the prothrombin solution gave a thrombin which may be regarded as relatively stable, since it suffered only a 15-20% deterioration in 4 hours (*cf.* supra[6]) and some 50-60% loss in 24 hours (at 10°C).

We have observed increased thrombin formation on adding prothrombin to thrombin solutions, and it seemed reasonable to attribute this to the action of excess Ca and thromboplastin in the thrombin preparation. Hence the prothrombin solution was heavily citrated with one-tenth volume of M/1 trisodium citrate. This necessitated

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[5] Glazko, A. J., and Ferguson, J. H., *J. Gen. Physiol.*, 1940, **24**, 169.

[6] Smith, H. P., *Am. Assn. Adv. Sci. Publ.*, 1940, **13**, 10.

[7] Ferguson, J. H., *J. Lab. Clin. Med.*, 1938, **24**, 273.

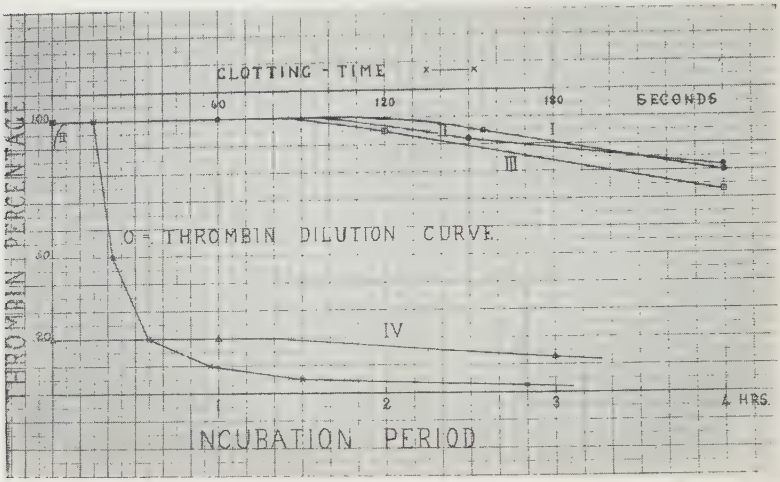

FIG. 1.

Thrombin content (relative) of aging thrombin and prothrombin mixtures. 10°C; pH 7.5.

a stronger $CaCl_2$ (N/2 instead of the usual N/10) for subsequent reactivation. There was no thrombin increase (curve IV) in the citrated mixture. Our confirmation of this Iowa finding lends further support to the view that prothrombin is *not* activated by thrombin and denies the so-called "autocatalytic" process, variously reported in the coagulation literature.

Taking the maximum thrombic potency (C.T. = 15 sec.) of full strength thrombin as 100% on our dilution scale (curve 0), 80% (four-fifths volume) of prothrombin solution, citrated as noted above, was mixed with 20% (one-fifth volume) of stable (75 min.) thrombin.

Data. Curve I is the prothrombin control made, be it noted, with citrated prothrombin containing saline, $CaCl_2$, and thromboplastin in the same amounts and proportions as the ingredients of the thrombic mixture used in curve III. Samples, removed at intervals, were maximally activated with additional Ca plus thromboplastin and the clotting-times converted into thrombin percentage by reference to the dilution curve. Curve II is the thrombin control. Both controls show a minor deterioration amounting to the loss of some 15-20% of activity in the 4-hour test period. Curve IV represents the thrombin content of the untreated mixture. It maintains the theoretical (20%) level for an hour or more and gradually falls off to some 13% in 3 hours. Curve III is the total thrombin yield (prothrombin plus thrombin) on reactivating samples of the mixture with additional $CaCl_2$ and thromboplastin. The theoretical level (100%) is present at the start, and the fall to 95% in 2½ hours and 75% in 4 hours is again of a minor order, quite unlike the dramatic prothrombin disappearance reported in the Iowa experiments.[6]

Discussion. It is concluded that thrombin and (citrated) prothrombin can co-exist with no more destruction of either than can readily be attributed to the natural instability of both preparations. Thrombin itself is neither an activator nor a destroyer of prothrombin. The lytic factor is an independent entity which, from previously reported data,[5] we believe to be an enzyme factor, probably serum tryptase.

11898 P

Influence of Sulfathiazole Therapy on Plasma Lipids in Pneumonia.*

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Previous investigations[1, 2] by the author have shown that during the febrile periods of acute infections such as pneumonia, there is a definite fall in the values for total and ester cholesterol, total fatty acids and phospholipids. The iodine numbers of the total fatty acids and of the phospholipid fatty acids also are lowered.

Further studies[3, 4] have revealed the interesting fact that the early administration of specific serum for pneumonia in children leads to a critical drop in the fever with rapid recovery which prevents the development of any significant changes in the levels of the plasma lipids. On the other hand, chemotherapy with sulfapyridine also causes a fall in the fever with improvement in the patient's condition but with little or no influence on the lipids of the serum which continue to undergo changes similar to those found in untreated cases of pneumonia. This latter observation raises the question as to whether

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* This work was supported by a grant from the Medical Research Fund of the University of Minnesota.

[1] Stoesser, A. V., and McQuarrie, Irvine, *Am. J. Dis. Child.*, 1935, **49**, 658.

[2] Stoesser, A. V., *Am. J. Dis. Child.*, 1938, **56**, 1215.

[3] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 168.

[4] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 201.

the response to all forms of chemotherapy is the same. Sulfathiazole, the thiazole analogue of sulfapyridine, was therefore investigated.

Six children ranging in age from 6 to 13 years were selected. All of the subjects were ill with pneumonia due to the pneumococcus. They received 0.3 g per kg of body weight per day of sulfathiazole for the first 48 hours after admission to the hospital and 0.15 g thereafter. The drug was administered orally every 4 hours, 6 times per day. It was well tolerated by all of the children. The first sample of blood for lipid determinations was obtained just before the sulfathiazole was started. At this time each child had been ill from one to 2 days and the fever was high, ranging between 39.5 and 40.4°C. The second sample was collected about 24 hours after critical drop in temperature to normal. The third and fourth blood samples were obtained on the fourth and seventh days of convalescence respectively. The sulfathiazole was discontinued on the morning of the fourth day of normal temperature just after the third sample had been drawn. Bloor's methods[5, 6, 7] were used to determine the total, ester, and free cholesterol values. The microgravimetric technic of Wilson and Hansen[8, 9] was employed in studying the other serum lipids. The Rosenmund-Kuhnhenn method as described by Yasuda[10] was used to determine the iodine absorption of the serum fatty acids.

The results are summarized in Table I.

The pneumonic infections in the 6 children were similar in nature. The cholesterol values were already a little lower than normal before sulfathiazole was started. The lowest level was reached in case 3. Following the administration of the drug, 4 of the subjects experienced a precipitous drop in the temperature to normal in 24 hours. In cases 2 and 3 the temperature did not reach the normal level until 48 hours after the onset of the drug therapy. After one day of no fever, the second blood sample was collected and the cholesterol esters were found to have risen to within the normal range in all except case 3. The roentgenograms revealed beginning resolution in 4 cases. On the fourth day of convalescence it was almost complete in all subjects. There was little change in the cholesterol values. By the seventh day, the roentgenograms showed complete resolution. The total cholesterol and esters were normal.

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[5] Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

[6] Bloor, W. R., and Knudson, Arthur, *J. Biol. Chem.*, 1916, **27**, 107.

[7] Bloor, W. R., personal communication to the author.

[8] Wilson, W. R., and Hansen, A. E., *J. Biol. Chem.*, 1936, **112**, 457.

[9] Hansen, A. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 376.

[10] Yasuda, M., *J. Biol. Chem.*, 1931-32, **94**, 401.

TABLE I.
Plasma Lipids of Pneumonia Before, During and After Administration of Sulfathiazole.

Case No.	Total cholesterol				Cholesterol esters				Free cholesterol			
	Mg per 100 cc serum											
	A	B	C	D	A	B	C	D	A	B	C	D
1	153	234	229	253	107	158	167	211	46	76	62	42
2	189	231	283	294	140	145	201	211	49	86	82	83
3	143	169	167	192	83	86	100	107	60	83	67	85
4	192	189	219	208	108	105	141	143	84	84	78	65
5	176	252	276	297	104	176	204	208	72	76	72	89
6	173	227	208	204	110	152	120	130	63	75	88	74
Avg	171	217	230	241	108	138	156	168	62	80	75	73

	Total fatty acids, mg %				Iodine No. of total fatty acids				Phospholipids, mg %				Iodine No. of phospholipid fatty acids			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	285	419	380	415	102	102	107	108	92	122	113	130	111	105	108	109
2	352	426	424	465	108	105	106	106	93	99	130	147	117	115	113	115
3	268	283	284	366	96	104	98	93	104	92	91	120	101	112	107	106
4	375	368	353	334	101	103	112	112	136	139	119	119	110	111	118	116
5	406	627	645	631	96	94	96	90	130	164	165	160	106	109	113	104
6	325	405	420	433	101	103	99	97	109	130	125	125	112	110	112	106
Avg	335	421	417	440	100	101	103	101	111	124	123	133	109	110	112	109

A—Blood sample collected before sulfathiazole was started.
B— ” ” ” after 24 hours of normal temperature.
C— ” ” ” on 4th day of convalescent period.
D— ” ” ” ” 7th ” ” ” ”

The total fatty acids and the phospholipids were depressed before the administration of sulfathiazole. The total fatty acids rose rapidly following the critical fall in temperature induced by the drug; the phospholipids returned to normal more slowly. The iodine numbers of the total fatty acids and of the phospholipid fatty acids underwent little change. The response to sulfathiazole was similar to that of specific serum therapy. This observation is significant for it may associate the fall and rise of plasma lipids with the appearance of immune bodies. In serum therapy the administration of antibodies produces a crisis and the serum lipids rise rapidly to within the normal range. In sulfathiazole and sulfapyridine therapy a critical drop in temperature occurs but only with the former drug is there a concomitant return to normal of the plasma lipids and Kneeland[11] has shown that a greater stimulation of the immune mechanism exists in the patient treated with sulfathiazole.

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[11] Kneeland, Yale, and Mulliken, Barbara, *J. Clin. Invest.*, 1940, **19**, 735.

11899

Biological Investigations with Radioactive Calcium and Strontium.*

CHARLES PECHER.† (Introduced by John H. Lawrence.)

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Radioactive calcium and strontium produced by the bombardment of these elements with 16 million volt deuterons have been used as tracers for mineral metabolism studies. As has been shown by Walke, Thompson and Holt,[1] Ca^{45} is the only radioactive calcium suitable for this purpose. Unfortunately its yield is low, and it emits a very soft beta-radiation (maximum energy 200 kev). Satisfactory measurements have been obtained with a screen wall counter described by Libby,[2] but the activity determinations are extremely time-consuming. This is a serious handicap in biological experiments, where the number of samples to be measured is generally very large. By this method, the fate of radio-calcium introduced into the stomach of a rat has been described by Campbell and Greenberg.[3]

A radioactive form of strontium (Sr^{89}), described first by Stewart, Lawson and Cork,[4] is a very valuable isotope for biological purposes. Its half-life is 55 days, and it emits 1.5 million electron-volt beta-particles, no gamma-rays, and can be produced abundantly by the cyclotron. The yield is about 6 millicuries per milliampere hour when strontium oxide is bombarded with 16 million volt deuterons and slightly higher when metallic strontium is used. At the same time, an appreciable amount of long-lived (about 100 days) radioactive yttrium‡ is produced, *i.e.*, ten milligrams radium gamma-ray equiva-

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* We wish to express our gratitude to Professor E. O. Lawrence and Dr. John H. Lawrence, who gave us the opportunity to do this research in their laboratory. We are also very grateful to the crew of the cyclotron and to the members of the laboratory, especially Dr. J. G. Hamilton and Dr. L. A. Erf for their help and suggestions. We are extremely indebted to Professor W. F. Libby, who allowed us to use his screen wall counter and his private laboratory for activity measurements.

This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

† Fellow Belgian-American Educational Foundation.

[1] Walke, H., Thompson, F. C., and Holt, J., *Phys. Rev.*, 1940, **57**, 177.

[2] Libby, W. F., and Lee, D. D., *Phys. Rev.*, 1939, **55**, 245.

[3] Campbell, W. Wesley, and Greenberg, David M., *Proc. Nat. Acad. Sci.*, 1940, **26**, 3, 176.

[4] Stewart, Lawson, and Cork, *Phys. Rev.*, 1937, **52**, 901.

‡ This isotope is discussed in an issue of *Physical Review* (Pecher, Charles, 1940, **58**, 843) which appeared since this paper was submitted for publication.

ient for one milliampere hour of 16 million volt deuterons. It emits a gamma-radiation very similar to that of radium and thus may be substituted for radium. This radioactive yttrium has been independently observed by DuBridge and Marshall[5] in the systematic study of the radioactive isotopes of strontium, yttrium and zirconium.

Radioactive strontium is detected with an electroscope or an electrometer, and the accuracy of the determinations is much better than that for Ca^{45}. The chemical and physiological similarity of the two elements, calcium and strontium, suggested a comparison of their metabolism. Strontium metabolism has been studied with the idea of the possible application of the radioactive isotope to therapeutic bone irradiation.

I. *Uptake of Radioactive Calcium and Strontium in the Tissues.* Doses of 0.1 cc radioactive calcium lactate solution (0.8 mg calcium, 200 counts per sec.) and 0.1 cc of radioactive strontium lactate solution (1.6 mg strontium, 0.8 microcurie) were intravenously injected into adult white mice. After 24 hours, the animals were sacrificed and the distribution of the activity in the various tissues measured.

Fifty-eight percent of the dose of radioactive calcium (average of 30 mice) and 33% of the dose of radioactive strontium (average of 35 mice) were recovered in the skeleton after 24 hours. The activity in the different tissues (average of 15 mice for calcium and 25 mice for strontium) is given in Table I. For comparison, the activities of the tissues after intravenous injection of radio-phosphate are given (average on 5 animals).

TABLE I.
Per Cent of Dose per Gram Wet Weight.

	Bone	Muscle	Skin and Hair	Dig. Tract	Liver	Other Visceræ
Ca^{45}	22	0.33	0.20	0.36	0.12	0.23
Sr^{89}	12	0.17	0.15	0.23	0.07	0.13
P^{32}	5.2	1.4	0.75	1.3	3.0	2.1

The uptake of calcium was greater than the uptake of strontium, but the distribution among the different tissues was almost the same for the two elements. Both of them were concentrated in the skeleton; the activity of the soft tissues was negligible in comparison with that of the bones; the digestive tract had a noticeable activity; that of the liver was extremely low, and there was almost none in the fat. In groups of animals killed at different times after the intravenous injection of radioactive calcium or strontium lactate, it appeared that the activity of the skeleton had nearly reached its maximum at eight hours. When introduced into the stomach of adult mice, 13 to 30% of the ingested calcium and 6 to 14% of the ingested strontium were recovered in the skeleton after 2 days. Uptake of calcium and strontium in the skeleton following intravenous injection was found to be nearly 3 times as great as that following oral administration.

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[5] DuBridge, L. A., and Marshall, John, *Phys. Rev.*, 1940, **58**, 7.

II. *Distribution of Radio-Calcium and Radio-Strontium in the Skeleton.* The distribution of radio-calcium and radio-strontium in the skeleton has been studied by two methods: (1) activity determination of bones or fragments of bones, and (2) autoradiographs of bones (by placing the bone or section of bone on a photographic film).

Activities of shaft and trabecular bone of rats and rabbits recently injected with radioactive strontium lactate have been compared and found to be higher in the trabecular bone. The epiphysis retains more radioactivity than the diaphysis. The epiphysial line is clearly marked on several autoradiographs showing an accumulation of strontium there (Fig. 1). The radio-strontium activity of each bone of the skeleton was measured separately and compared with the weight of inorganic material of each bone. Activity was higher in the dorsal vertebrae (0.030% per mg) than in most of the other bones (average 0.018% per mg). Activity of the inferior maxilla was similar to the activity of the other bones (0.0175%). The average activity of the teeth was higher than that of the bones. The base of the incisor teeth (mice and rabbits) had an exceedingly high activity (0.141% per mg dry weight) (Fig. 3).

A selective concentration of the activity appears during the period of healing of fractured bones. The uptake of radioactive calcium in the healing tibia of a rat (fractured 2 weeks before) was 1.7% of the ingested dose of radio-calcium lactate (32 mg calcium), while the other tibia had an activity of only 0.4%.

III. *Excretion of Radioactive Calcium and Strontium.* Intravenously administered calcium and strontium are excreted both in the feces and in the urine. In mice the larger fraction was generally excreted in the feces. An appreciable activity was found in the contents of the small (.33% of dose) and large (.88% of dose) intestine 4 hours after the intravenous injection of radio-calcium lactate. No activity was detectable in the stomach. The exact ratio between the renal and intestinal excretion of calcium and strontium was difficult to determine exactly in mice because of the difficulty of completely separating the feces and the urine. For this reason rats were used, and the daily activity of feces and urine after subcutaneous injection of radioactive strontium lactate is given in Table II in percent of the dose. On the day following a large subcutaneous dose, most of the

FIG. 1.

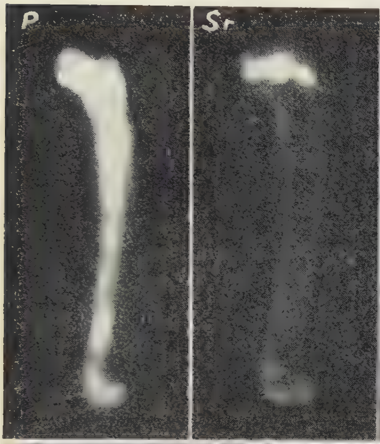
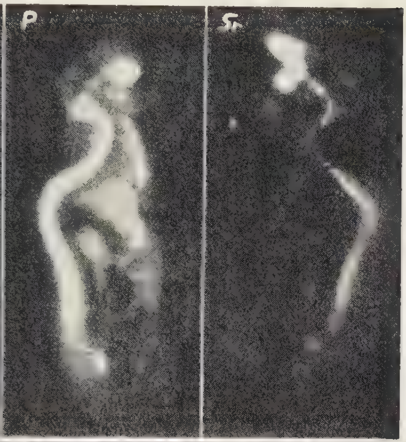


FIG. 2.



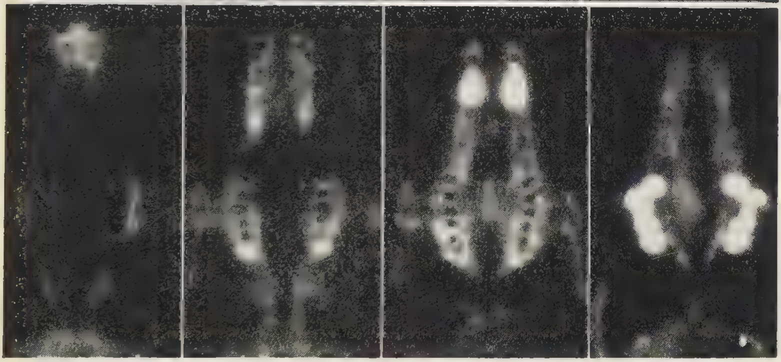


FIG. 3.

FIG. 1. Autoradiographs of the humeri of rabbits five days after intravenous administration of radio-phosphate (A) and of radio-strontium lactate (B). The bone marrow is present in both preparations, and the beta-ray activities of both bones are approximately the same.

FIG. 2. Autoradiographs of whole mice 60 hours after intravenous administration of radio-phosphate (A) and radio-strontium lactate (B). In both pictures the bones and the root of the teeth are strongly radioactive. In A the soft tissues and especially the liver have a high activity. In B the soft tissues contain almost no radioactivity. The intestine contains no radio-phosphorus in A, but in B the colon appears because of the presence of strongly active feces.

FIG. 3. Autoradiographs of successive sections (inferior section to left, superior to right) of the superior maxilla of a rabbit, five days after administration of radioactive strontium lactate showing the distribution of the radioactivity in the various levels in the teeth. The high uptake by the roots is evident.

activity was found in the urine. A few days later about the same amounts were excreted in the feces and urine. The secretion of strontium and calcium in milk will be described in a later paper on the metabolism of strontium during pregnancy.[6]

TABLE II.
Excretion in Feces and Urine of Intravenously Administered Radioactive Strontium Lactate.
(Per cent of dose.)

Rat No.		1st	2nd	3rd	4th	5th	6th	8th day
			Dose: 17 mg Sr.					
47	Urine	18.5	3.36	1.61	.77	.50	.41	.28
	Feces	6.15	7.80	1.85	.63	.54	.45	.20
49	Urine	26.4	4.40	1.37	.71	.33	.45	
	Feces	11.7	13.3	1.52	1.28	.52	.79	
54	Urine	37.8	4.21	2.58	1.38	.95	.64	.26
	Feces	9.65	11.2	5.00	2.22	1.20	.58	.30
			Dose: 2.3 mg Sr.					
50	Urine	13.8	1.6	0.675	.55	.27	.44	.22
	Feces	9.8	6.8	1.67	1.47	1.50	.23	.18
56	Urine	20.8	3.66	0.98	.39	.35	.14	.07
	Feces	7.13	14.0	2.79	.65	.34	.19	.12
58	Urine	20.3	6.17	2.97	.78	.82	.67	.57
	Feces	1.32	6.32	7.21	3.53	1.22	.82	.54

IV. *Comparison of the Uptake of Strontium Chloride, Lactate and Gluconate.* Isomolecular solutions of radioactive strontium chloride, lactate and gluconate were prepared (15 mg strontium per cc). Doses of 0.1 cc of each solution were injected intravenously in 3 groups of 9 adult mice. For 3 other groups of 9 mice, doses of 0.2 cc were introduced in the stomach. After 48 hours, the animals were killed, and the activity of their skeletons measured separately. The average values and the ranges are given in Table III in percentage of the dose fixed in the skeleton.

No significant difference appears whether the strontium is given in chloride, lactate, or gluconate for either method of administration.

Discussion. Tracer studies with radioactive calcium and strontium have shown a close analogy in their metabolism. Although the retained fraction is higher for calcium than for strontium, the distribution of the two elements in different tissues is very similar, the most striking fact being a high uptake in the skeleton. Our observations of a higher concentration in trabecular than cortical bone agrees with the views of Bauer, Aub, and Albright.[7]

TABLE III.
Per Cent of Dose Fixed in the Skeleton in 9 Adult Mice.

	Intravenously			Orally		
	Sr Chloride	Sr Lactate	Sr Gluconate	Sr Chloride	Sr Lactate	Sr Gluconate
Avg	33.1	34.0	33.0	10.2	11.9	10.3
Range	(27.8-40.7)	(22.0-45.5)	(26.6-36.8)	(7.0-15.3)	(3.7-18.0)	(8.5-12.3)

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[6] Erf, L. A., and Pecher, Charles, in press.

[7] Bauer, W., Aub, J. C., and Albright, F., *J. Exp. Med.*, 1929, **49**, 145.

The comparison of the uptake of chloride, lactate and gluconate of strontium shows that the difference, if any, is much smaller than the individual variations. If there is any difference in the uptake of calcium chloride, lactate and gluconate, this difference will probably be of minor importance. This fact can be related to the observation of Greenberg and Gunther[8] that the blood calcium in dogs increases to the same extent whether the same dose of calcium is ingested in chloride, lactate or gluconate. The observation that the uptake in the skeleton is about 3 times higher after intravenous than after peroral administration suggests that approximately one-third of the ingested dose of a calcium or strontium salt is absorbed by the intestine. Strontium and calcium are excreted both in the feces and in the urine.

The selective fixation of radio-strontium in the skeleton, its fairly good yield, the suitable energy of its beta-rays, its convenient half-life and the innocuousness of small doses[9] have given a specific method of irradiation of the skeleton.

Summary. The metabolism of calcium and strontium has been studied on normal animals with the radioactive isotopes of those elements. Uptake in the skeleton of different salts of strontium has been compared. These experiments have furnished two practical findings: (1) a method to irradiate the skeleton selectively for therapeutic purposes, (2) the secondary production of appreciable amounts of a long-lived radioactive yttrium, the most likely among the artificial radioactive elements now known, which may be substituted for radium as a penetrating gamma-ray source.

11900

Radio-Calcium and Radio-Strontium Metabolism in Pregnant Mice.*

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Studies of calcium and strontium metabolism in mice with radioactive isotopes have shown a selective concentration of these elements in the skeleton.[1] It was of interest to determine how pregnancy affects this metabolism and to what extent calcium and strontium, previously fixed in the skeleton of the mother, would migrate to the foetus and later to the milk.

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[8] Greenberg, D. M., and Gunther, L., *Arch. Int. Med.*, 1932, **50**, 855.

[9] MacCance, A., and Widdowson, E. M., *Biochem. J.*, 1939, **33** II, 1822.

* We wish to express our gratitude to the members of the Radiation Laboratory and especially to Drs. John H. Lawrence and L. A. Erf for their help and suggestions. This investigation was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

† Fellow of the Belgian-American Educational Foundation.

Methods. Adult virgin and pregnant mice were injected with doses of 0.1 cc radioactive calcium lactate solution (containing 0.8 mg Ca and emitting radiation which produced about 200 counts/sec. with the Libby screen wall counter[2]) and radioactive strontium lactate solution (containing 1.6 mg Sr and emitting radiation equivalent to about one microcurie).‡ Throughout, the animals received a normal diet containing about 1% of $CaCO_3$. At different periods after the injection the mice were sacrificed and the radioactivity of bones, soft tissues and uterus of each mouse was measured separately. Assays of radio-strontium were determined with a DuBridge electrometer and those of radio-calcium with a Libby screen wall counter.[2] Aliquots of the injected dose were used as reference standards. The probable error for these measurements is ±3% for radio-strontium and ±10% for radio-calcium. Newborn mice were killed within 12 hours after birth and the radioactivity of their whole bodies assayed. On some samples, total calcium was determined and the specific activities calculated. In a few cases, newborn mice containing no radioactivity were given to "radioactive mothers," so that the amount of radioactivity transferred from the mothers to the adopted offspring through the milk could be determined.

Results and Discussion. The uptake in mothers, foetuses and offspring of radio-strontium and radio-calcium injected previous to or during pregnancy is given in Table I. It appears, as has been previously observed, that a few days after injection, most of the radioactive calcium or strontium is fixed in the bones, while the activity of the soft tissues and that of the uterus is low. Nevertheless, at birth there is an appreciable amount of radio-calcium or radio-strontium in the offspring. In the radio-calcium experiments, the specific activity of the whole body of the offspring was frequently higher than the specific activity of the mother's bone, although the radioactive calcium of the newborn mice originates mostly from the mothers' bones. Specific activities were 0.49, 0.36, 0.52 and 0.19% of the

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[1] Pecher, Charles, PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 86.

[2] Libby, W. F., and Lee, D. D., *Phys. Rev.*, 1939, **55**, 245.

‡ These "tracer" activities of strontium and calcium were kept small enough to avoid an effect of the radiation on metabolism.

TABLE I.*
Distribution of Radioactive Ca and Sr in Mothers, Foetuses, and Offsprings at Different Periods After the Administration of the Labelled Isotopes.
(Percent of dose.)

Days between inj. and killing†	State of‡ pregnancy when killed	Mother's bones	Mother's soft tissues	Foetuses and uterus	Offspring	Accumulated in young mice during lactation period
Experiment with Radio-strontium.						
2	n.p.	20.0	1.0	0.0		
2	p.III	17.5	1.5	0.11		
1	p.III	19.0		0.35		
4	n.p.	34.0		0.0		
4	p.I	24.1	0.9	0.0		
9	p.II	20.1		0.0		
9	12 off	18.5			2.65	
9	7 off	25.9			1.75	
9	10 off	15.0			3.0	
11	9 off	31.7			2.45	
11	9 off	12.9			1.54	
12	10 off	13.6			2.04	
12	10 off	15.1	1.0		3.2	
14	7 off	24.0			1.55	
15	8 off	20.7			1.49	
21	p.IV	13.0	0.13	0.024		
21	n.p.	13.9	0.10	0.0		
21	5 off	10.0	0.10		1.4	
21	6 off	12.2	0.15		1.9	
21	5	11.6	0.14		0.44	
45	6 off				0.28	
	Lact 20d	11.8				1.0
45	9 off				0.80	
	Lact 20d	13.0				0.80
45	6 off				0.18	
	Lact 7d	7.5				0.34
20	Lact 8d	7.9				2.15
Experiment with Radio-calcium.						
1	n.p.	64.0	3.3	0.0		
1	n.p.	58.0		0.0		
1	p.IV	48.0	3.0	2.8		
10	p.IV	70.0	<1.2	1.8		
10	8 off	65.0	<1.2		16.3	
11	9 off	57.0	<2.0		3.8	
12	9 off	53.0			7.0	
20	8 off	28.0	<1.2		5.8	

*Each line of figure represents values from individual mice.

†The duration of pregnancy in mice is 21 days.

‡p.—pregnant; n.p.—not pregnant; I, II, III, etc.—1st, 2nd, 3rd, etc., quarter of the pregnancy; off.—offspring; lact.—lactation with duration in days of the lactation period.

administered dose per milligram of calcium for the newborn mice and 0.26, 0.26, 0.25 and 0.20% respectively for the mothers' skeletons (Exp. 9, 17, 18). These observations suggest that the last calcium fixed in the mothers' bones is the first to be removed.

When offspring containing no radioactivity were given to "radioactive mothers," a very appreciable amount of activity was found in the adopted offspring after a few days (Table I). This demonstrates migration of these elements from the mother's skeleton to the offspring through the milk.

The immediate secretion of strontium in the milk was studied by injecting 0.1 cc radioactive strontium lactate solution intravenously into lactating mice; after 2 days, 19, 20 and 11.8% of the dose was recovered in the offspring. The immediate secretion of radioactive strontium in milk of 2 cows has also been studied.[3] During the 4 days following injection, 11 and 7.8% of the administered dose of radioactive strontium lactate (0.85 g of metallic strontium) was recovered in the milk.

In order to determine if the migration of the radioactive mineral salts from the mother's bones to the foetus and to the milk would alter the rate of excretion of radioactive strontium, the excreta of 6 mice previously injected with radioactive strontium were assayed for a period of one month. Three out of the 6 mice became pregnant. A slight increase (from 0.1-0.2% to 0.2-0.4% of the dose) in the daily rate of excretion of radioactive strontium occurred during the last 3 or 4 days of pregnancy, and particularly during the first 10 days of the lactation period.

Conclusion and Summary. Radioactive strontium and calcium have been used to study the mineral metabolism in mice during pregnancy. It appears that part of the calcium and strontium previously fixed in the skeleton of mice migrates to the foetus during the last days of pregnancy and to the offspring through the milk. When radioactive strontium lactate is injected intravenously into lactating mice and cows, appreciable amounts are excreted in the milk.

11901

Creatine-Creatinine Excretion in Neuromuscular Diseases Treated With Alpha-tocopherol and With Testosterone.*†

WALTER FLEISCHMANN. (Introduced by E. A. Park.)

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Deficiency of vitamin E in guinea pigs and rabbits results in dystrophy of the musculature which in some ways resembles muscular dystrophy in human beings. Mackenzie and McCollum[1] have shown that the nutritional muscular dystrophy of rabbits is readily cured by administration of alpha-tocopherol. These authors have studied the excretion of creatine and have found that increased creatinuria invariably attends the deficiency in rabbits and may precede the gross symptoms by 2 weeks or more. A marked reduction in urinary creatine occurs within 24 or 48 hours of vitamin E administration. The antidystrophy requirement of the rabbit for alpha-tocopherol does not exceed 1.0 mg per kilo per day. Verzar[2] has shown that rats develop creatinuria together with muscular dystrophy when put on a vitamin E-free diet. The creatinuria of these animals was suppressed by large doses of alpha-tocopherol. Fifty to 100 mg daily were necessary to reduce the creatine to normal values. When tocopherol therapy was discontinued creatine appeared again in the urine within 24 hours. Verzar believes that alpha-tocopherol is concerned with the fixation of the creatine in the body.

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[3] Erf, L. A., and Pecher, Charles, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 762.

* Supported by a grant from the Commonwealth Fund for Endocrine Research in Children.

† The alpha-tocopherol was kindly supplied by Merck & Co., Inc., Rahway, N. J.

A number of workers have recently tried treatment with vitamin E in neuromuscular disorders in human beings. Bicknell[3] claims clinical improvement in 12 out of 13 cases of progressive muscular dystrophy, 2 cases with amyotrophic lateral sclerosis and one case of amyotonia congenita treated with wheat germ. Wechsler[4] reports clinical improvement in 2 early cases of amyotrophic lateral sclerosis in which synthetic alpha-tocopherol was employed. Stone[5] thinks that clinical improvement was obtained in 5 cases of progressive muscular dystrophy by adding wheat germ oil to the diet. Quite recently Shelden, Butt and Woltmann[6] reported negative results in 8 cases of progressive muscular dystrophy, 4 cases of progressive muscular atrophy, 6 cases of amyotrophic lateral sclerosis treated with alpha-tocopherol. All of these studies are based on clinical observations. Milhorat, Weber and Toscani[7] found that in 2 cases of dermatomyositis prolonged administration of 125 g of wheat germ daily was followed by decrease in creatinuria, increase in urinary creatinine and definite clinical improvement. As far as we are aware no biochemical studies have been reported on cases of progressive muscular dystrophy or amyotonia congenita treated with vitamin E.

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[1] Mackenzie, C. G., and McCollum, E. V., *Science*, 1939, **89**, 370; *J. Nutrition*, 1940, **19**, 345.

[2] Verzar, F., *Schweiz. Med. Wochenschr.*, 1939, **69**, 738.

[3] Bicknell, F., *Lancet*, 1940, **1**, 10.

[4] Wechsler, I. S., *J. A. M. A.*, 1940, **114**, 948.

[5] Stone, S., *J. A. M. A.*, 1940, **114**, 2187.

[6] Shelden, C. H., Butt, H. R., and Woltmann, H. W., *Proc. Staff Meetings Mayo Clinic*, 1940, **15**, 577.

[7] Milhorat, A. T., Weber, F. C., and Toscani, V., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 470.

Increased creatinuria has been found in many of the conditions in which there is atrophy of the musculature. It has been described in progressive muscular dystrophy, amyotonia congenita and amyotrophic lateral sclerosis. In these conditions the creatinuria is associated, as a rule, with a lowered ability to retain ingested creatine and with a subnormal output of creatinine. (Hunter,[8] Milhorat and Wolff.[9])

Report of Study. Treatment with alpha-tocopherol was tried in 2 cases of progressive muscular dystrophy and in one case of amyotonia congenita (Oppenheim's disease) in the Harriet Lane Home. In addition, the effect of testosterone on creatine-creatinine metabolism was studied in one case of amyotonia congenita and one case of muscular dystrophy; it has been shown that treatment with testosterone brings about a decrease of the amount of creatine excreted by castrate male rats (Kun and Peczenik[10]) and by human eunuchoids (Kenyon and coworkers[11]).

The patients were put on a diet low in creatine and 24-hour specimens of urine collected. In one patient (E.W.) there were nearly always losses in collecting the urine, so that in this case only the ratio of creatine to creatinine is significant, not the absolute amounts found in the urine. Creatine tolerance tests were made by giving 1.32 g of creatine by mouth in the morning and estimating the amount of this exogenous creatine excreted during the first 24 hours. The exogenous creatine not excreted within this time is considered retained and expressed in percent of the ingested amount. Creatine and creatinine were determined by the method of Folin.

The results are summarized on Table I. In no case was any clinical improvement observed as result of the treatment.

Conclusions. Neither alpha-tocopherol nor testosterone-propionate in the doses given had any effect on the creatine-creatinine excretion in the cases of amyotonia congenita and progressive muscular dystrophy studied. This should not be interpreted as failure of these substances as therapeutic agents. It indicates, however,

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[8] Hunter, A., *Creatine and Creatinine*, Longmans, Green and Co., Ltd., London, 1928.

[9] Milhorat, A. T., and Wolff, H. G., *Ann. Int. Med.*, 1936, **9**, 834.

[10] Kun, H., and Peczenik, O., *Pflugers Arch.*, 1935, **236**, 471.

[11] Kenyon, A. T., Sandiford, I., Bryan, A. H., Knowlton, K., and Koch, F. C., *Endocrinol.*, 1938, **23**, 135.

TABLE I.

Avg daily excretion of			
Creatine mg	Total† Creatinine mg	Creatine in % of total %	Treatment
L.M., ♂, 7 yrs, 30 kg, progressive muscular dystrophy.			
207	427	50	11 days without treatment.
196	372	54	6 ’’ 100 mg alpha-tocopherol‡ daily parenterally.
198	453	44	5 days without treatment.
J.W., ♂, 10 yrs, 22 kg, progressive muscular dystrophy.			
203	383	53	15 days without treatment.
208	373	56	19 ’’ 110 mg alpha-tocopherol daily by mouth.‡
280	492	57	6 days without treatment.
242	414	58	8 ’’ 220 mg alpha-tocopherol and 15 cc liver extract daily by mouth.
122+*	245+	50	14 days 25 mg testosterone-propionate twice weekly parenterally.
E.W., ♀, 3½ yrs, 12 kg, amyotonia congenita.			
89+	120+	74	19 days without treatment.
78+	112+	70	20 ’’ 200 mg alpha-tocopherol daily parenterally.‡
90+	127+	71	14 days without treatment.
D.S., ♂, 6 yrs, 50 kg, amyotonia congenita.			
382	639	60	28 days without treatment.
354	572	62	14 ’’ 50 mg testosterone-propionate twice weekly parenterally.
390	611	66	16 days without treatment.

*Losses in urine collection; only the creatine in % of total is significant.

†Creatine plus creatinine.

‡Alpha-tocopherol dissolved in olive oil when given by mouth and in peanut oil when given parenterally.

Creatine tolerance tests on patient L.M. showed 41% retention before treatment, 21% retention after treatment with alpha-tocopherol. The tests on patient J.W. showed 40% retention before treatment, 45% retention after the first 19 days of treatment with 110 mg alpha-tocopherol by mouth.

that alpha-tocopherol does not have the same inhibitory effect on creatinuria in neuro-muscular disturbances in human beings as in the nutritional muscular dystrophy in rabbits. This suggests a fundamental difference between nutritional muscular dystrophy in rabbits and progressive muscular dystrophy, amyotonia congenita and similar neuromuscular disturbances in human beings.

11902

Comparative Depressant Action of Pentothal and Thioethamyl.

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For short operations in both civil[1] and military[2] practice, intravenous barbiturate anesthesia is becoming increasingly more popular. Rapidly metabolized barbiturates or thiobarbiturates are employed for this purpose. Of the thiobarbiturates, those used include sodium ethyl-l-methylbutyl thiobarbiturate ('pentothal') and sodium isoamylethyl thiobarbiturate ('thioethamyl'). The former was reported as an useful intravenous anesthetic agent by Lundy and Tovell and the latter by Cullen and Rovenstein and from the point of view of the anesthetist there appears to be little to choose between the two.[1] Chemically the two compounds are almost identical. Most pharmacological studies have been concerned with their relative anesthetic power, toxicity and effect on blood pressure and respiration, again with results similar in the two drugs.[3] The present report describes a study of the effect of equal doses of 'pentothal' and 'thioethamyl' on several other organs.

The methods employed were the more or less standardized pharmacological procedures similar to those described in Jackson's Experimental Pharmacology. Increasing doses of one or other thiobarbiturate were administered until a dose was reached which produced a definite depression of the system under investigation. The effect of this dose of the two thiobarbiturates was then compared. The systems studied and the doses employed in each case were as follows: (a) the hind limb flexor reflex time in brain-pithed frogs given 2 mg of the drugs into the dorsal lymph sac; (b) the suspended frog heart bathed with a 0.1% solution in buffered Ringer's solution of the drugs; (c) the isolated frog gastrocnemius muscle assembled on a Starling myograph and bathed with a 0.1% solution of the drugs in buffered Ringer; (d) the respiratory rate and depth in tracheal cannulated urethanized rabbits given 15 mg of the drugs intravenously; (e) isolated rabbit small bowel segments assembled in a Magnus bath containing 0.01% of the drugs in Locke's solution;

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[1] Tovell, R. M., and Garofalo, M., *N. Y. State J. Med.*, 1939, **39**, 2036.

[2] British Army: Jeffrey, J. S., *Edin. Med. J.*, 1940, **47**, 727; German Army: Krabbel, M., *Med. Klin.*, 1939, **35**, 1591.

[3] Gruhzit, O. M., Dox, A. W., Rowe, L. W., and Dodd, M. C., *J. Pharm. and Exp. Therap.*, 1937, **60**, 125.

TABLE I.
Depressant Effect of Pentothal and Thioethamyl.

Function	% depression (Mean ± standard error)	
	Pentothal	Thioethamyl
Spinal Reflex, Frog	90 ± 16	85 ± 13
Skeletal Muscle Contraction, Frog	53 ± 6	46 ± 5
Cardiac Rate, Frog	42 ± 5	29 ± 3
Respiratory Rate, Rabbit	21 ± 6	10 ± 6
Respiratory Depth, Rabbit	11 ± 11	18 ± 10
Isolated Bowel Tonus, Rabbit	14 ± 9	7 ± 8
do. Strength of Contractions	65 ± 7	41 ± 7
Isolated Uterus Tonus, Guinea Pig	34 ± 9	25 ± 7
Urine Volume, Rabbit and Cat	33 ± 9	28 ± 5
''Grand Mean Depression''	40 ± 9	32 ± 7

(f) isolated guinea pig uterine horns assembled in a Magnus bath containing 0.01% of the drugs in Locke's solution; (g) urine volume per minute in bladder cannulated, urethanized rabbits and cats given 2 mg of the drugs intravenously. Fifteen to 20 animal experiments were performed in each case except in (d) and (g) in which 8 to 10 experiments were done. The percentage depression of the reaction being studied was determined, then the mean percentage depression of all experiments of the group, its standard deviation and standard error.

The results have been summarized in Table I. The depressant effect of thioethamyl was found to be of the same order as that of pentothal in all cases. In 8 out of the 9 comparisons made, pentothal proved to be slightly more depressant on the average than thioethamyl but the differences were not marked for measurements of this type. In no case was there a statistically significant difference between the means. These experiments therefore indicate that thioethamyl and pentothal have similar depressant effects in corresponding doses upon spinal cord reflexes, skeletal muscle, the heart, respirations, small intestine, uterus and urine formation.

11903

Relation of Dietary Fat to Riboflavin Requirement of Growing Rats.*

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Studies in this laboratory on the relation of the level of fat, carbohydrate, and protein in the diet to the vitamin requirements of growing animals led to the observation that the feeding of high levels of fat to young rats increased the riboflavin requirement. Modifications of the basal riboflavin-low ration K_{21} described by Wagner, Axelrod, Lipton, and Elvehjem[1] were utilized in studying this effect. Lard was isodynamically substituted for dextrin at two levels so that in ration K_{22} the total fat content was about 25% and in K_{23} about 40%. Choline was added to the extent of 5 mg per 100 g of K_{21} or its isocaloric equivalent in the other rations. Storage of the rations in the refrigerator and daily feeding prevented rancidity.

In the first series albino rats weighing from 60 to 75 g were partially depleted of their riboflavin reserves by placing 12 rats on each of the above rations for a period of 4 weeks. Four animals in each group were then supplemented with 3γ of riboflavin per day, four, with 6γ of riboflavin, and four were continued on the basal ration with no additional supplements. The growth responses of typical animals are illustrated in Fig. 1.

The growth curves show that the feeding of high levels of fat to growing rats increases the requirement for riboflavin and that the higher the fat level, the more noticeable is the effect. The greater loss of hair and extent of dermatitis observed in the rats raised on the high fat diets were even more notable than the weight differences. Rats maintained in a chronic state of deficiency on the high fat rations developed a spastic gait due to a paralytic condition

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We are indebted to Merck and Company, Rahway, N. J., for supplies of thiamin, nicotinic acid, vitamin B_6, calcium pantothenate; to Abbott Laboratories, North Chicago, Ill., for haliver oil; and to Wilson Laboratories, Chicago, for liver extract.

[1] Wagner, J. R., Axelrod, A. E., Lipton, M. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **136**, 357.

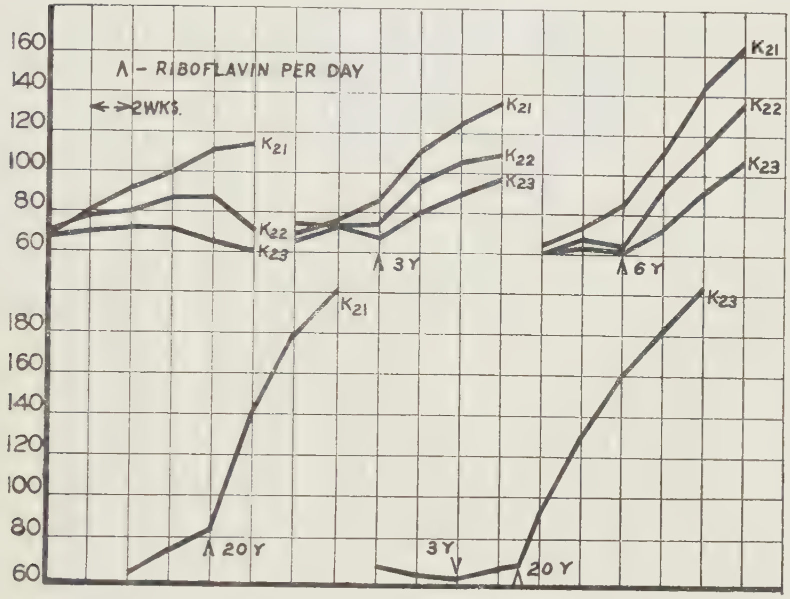

Fig. 1.

Growth curves for rats raised on rations containing different levels of fat and supplemented with different levels of riboflavin.

of the hind legs. This condition could be cured within 2 weeks after the administration of riboflavin. The characteristic high degree of deficiency and the spastic gait developed in a rat by feeding a high level of fat is illustrated in Plate 1. Cataracts similar to those described by Day, Langston, and O'Brien[2] were observed in several of these rats. Animals in the final stages of deficiency when supplemented with 20 or 40γ of riboflavin per day responded remarkably and developed a normal appearance and weight within 3 to 6 weeks. The growth curve of control rats receiving the high carbohydrate diet plus 20γ of riboflavin daily and the curve showing the cure effected by riboflavin in rats depleted on the high fat ration are given in Fig. 1.

Additional evidence supporting interpretation of the above data was gained by subjecting animals to a free choice of both high and low fat-containing rations. During the 10th week of the experiment 3 rats, each of which was receiving a different level of supplemented riboflavin, were selected from each group and fed both the high carbohydrate ration and the ration containing 40% fat. Food consumption records revealed that rats which had been raised on the

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[2] Day, P. L., Langston, W. C., and O'Brien, C. S., *Am. J. Ophth.*, 1931, **14**, 1005.

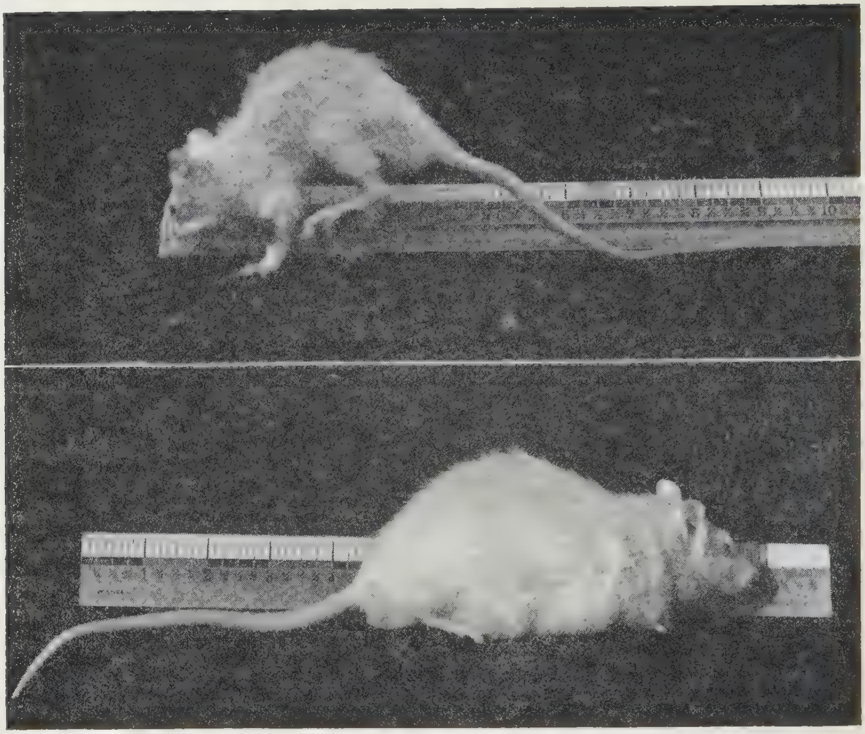

PLATE 1.

The upper photograph shows a rat raised for 18 weeks on the ration containing 40% fat plus 3 γ of riboflavin per day. Weight: 66 g. Below is the same animal 4 weeks later after having been fed 40 γ of riboflavin daily during this period. Weight: 205 g.

high fat rations selected the high carbohydrate ration in preference to the high fat ration with almost complete neglect of the latter. A corresponding weight increase resulted. Though rats which had been receiving the high carbohydrate diet selected the high fat ration during the first 3 or 4 days of the experiment, they gradually reversed their choice until they were consuming only the high carbohydrate ration. These animals were then given only the high fat ration and a retardation of growth followed. That the selection was not influenced by rancidity or the greasy texture of the high fat ration was shown by the preference of normal rats for the fatty diet.

A second series of albino rats weighing from 30 to 40 g were partially depleted for 2 weeks on rations K_{21} and K_{22} in which the choline and vitamin B_6 had been doubled and crystalline pantothenic acid added at the level of 500γ per 100 g ration. At the end of the depletion period the rats which had been fed the high fat ration weighed less than those which had been placed on the high carbohydrate ration. Various levels of riboflavin were then added, namely, 0, 6, 18, and 54γ per day. The final weights of the animals after being on experiment for 7 weeks and the gains in weight during the 5-week supplementation period are given in Table I. Each figure represents an average of 3 rats. Rats receiving no added riboflavin attained a higher weight plateau level when fed the high carbohydrate diet than when fed the high fat diet as is shown by the final weights and gains in weight per week. All animals supplemented with 6γ of riboflavin daily showed a weight response to the vitamin but rats fed the high fat ration showed a smaller weekly weight increase and a correspondingly lower final weight. The differences between rats receiving the high and low fat rations plus 18γ of riboflavin daily were not large but might indicate that this level of riboflavin is not quite adequate for the support of maximum growth of animals fed the high fat ration. Both rations supported good growth in rats receiving 54γ of riboflavin daily.

From a number of possible explanations for the antagonistic effect of fat on riboflavin, two deserve special consideration; namely, (1) increasing the level of fat in the diet may alter the intestinal flora so that less than normal bacterial synthesis, or more than normal bacterial destruction of riboflavin occurs, or (2) riboflavin may be directly concerned with fat metabolism or phosphorylation. As yet no experimental evidence is available supporting definite conclusions regarding the mechanism involved. Further work in this direction is in progress at this laboratory.

TABLE I.
Response of Rats Raised on High and Low Fat Rations to Various Levels of Added Riboflavin.

	Micrograms of riboflavin per day			
	0		6	
Ration	Final wt, g	Gain* per wk, g	Final wt, g	Gain* per wk, g
K_{21}	84	4.7	112	11.5
K_{22}	61	2.3	86	8.7

	Micrograms of riboflavin per day			
	18		54	
Ration	Final wt, g	Gain* per wk, g	Final wt, g	Gain* per wk, g
K_{21}	180	26.2	185	26.9
K_{22}	166	23.3	193	29.5

*Based on the 5-week supplementation period.

In summary, it has been demonstrated that increasing the fat level in a riboflavin-low ration has a corresponding deleterious effect on the growth of young rats, and that the administration of adequate amounts of riboflavin completely corrects this deficiency.

11904

Reductions in Blood Pressures of Renal Hypertensive Dogs by Hog Renin.*†

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A number of attempts to reduce the blood pressures of renal ischemic hypertensive dogs have been reported recently. Most of these have been unsuccessful, although a few investigators have obtained results showing some promise. Thus Davis and Barker[1] reported that potassium sulphocyanate reduced the blood pressure of renal hypertensive dogs. Mansfield, *et al.*,[2] found that nephro-splenopexy but not nephro-omentopexy caused a more or less permanent decrease in the blood pressure of dogs rendered hypertensive by the Goldblatt technic. Similar results were later reported by Cerqua and Samaan.[3] Obviously methods involving the production of an increase in the collateral circulation to the ischemic kidney have at best a limited applicability to the therapy of essential and malignant hypertension in the human since the ischemia in the clinical condition is ordinarily due to arteriosclerosis rather than narrowing of the renal arteries. Katz and coworkers[4] reported that transplanted autolyzing kidney tissue reduced the blood pressure of renal hypertensive dogs but Goldblatt[5] was unable to confirm this

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* This work was aided by a grant from the Graduate School Research Fund of the University of Illinois.

† We appreciate the technical assistance of Messrs. B. Gomberg and M. L. Goldberg.

[1] Davis, L., and Barker, M. H., *Ann. Surg.*, 1939, **110**, 1016.

[2] Mansfield, J. S., Weeks, D. M., Steiner, A., and Victor, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 708.

[3] Cerqua, S., and Samaan, A., *Clin. Sc.*, 1939, **4**, 113.

[4] Rodbard, S., Katz, L. N., and Sokolow, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 360.

[5] Goldblatt, H., personal communication.

finding. Harrison and collaborators[6] reported reductions in the blood pressure of renal hypertensive dogs and of humans with essential and malignant hypertension by means of a kidney extract containing the antipressor substance believed to be present in normal renal tissue. Because of untoward effects observed in a number of their dogs, as well as for other reasons, these authors reserved judgment relative to the ultimate specificity and value of this form of therapy. Page, *et al.*,[7] using a similar antipressor renal extract, reported favorable effects on the blood pressures of hypertensive dogs and humans. Both of these groups considered this antipressor substance to be different from any of the known depressor agents. However, more work is necessary to determine the comparative effectiveness of this type of renal extract and other antihypertensive agents, as well as its specificity for experimental renal hypertension and clinical essential or malignant hypertension.

Several months ago we reported the successful production of an antiserum for dog renin in the rabbit and for hog renin in the dog.[8] The active principle of the antiserums, which we designated "antirenin", was shown to neutralize the acute pressor effect of the antigenic renins. We have since produced an antiserum for rabbit renin in the guinea pig and for hog renin in the rabbit. We also noted that rabbit antiserum for dog renin was somewhat less effective in neutralizing rabbit renin. Since then we have found that the dog antiserum for hog renin is likewise slightly less effective in neutralizing dog renin.

In view of the increasing possibility that renin is the pathogenetic pressor agent in experimental renal ischemic hypertension, we stated[8] that, among other things, we purposed to determine the value of antirenin, actively produced, in the therapy of experimental renal hypertension. At this time, therefore, we present preliminary results with the treatment of renal ischemic hypertension in dogs by injections of hog renin.

Methods. Four dogs were rendered hypertensive by the Goldblatt technic and the resulting hypertension was allowed to stabilize over a period of 3 to 23 months before hog renin injections were begun. Mean blood pressure readings were obtained by femoral artery puncture 2 or 3 times per week. Blood urea nitrogen studies, urinalyses, and body weight determinations were made at biweekly intervals. The 4 hypertensive dogs were treated with daily intramuscular injections of hog renin for 4 months each. The renin solution was equivalent to 1 g of fresh kidney cortex per cc and was administered in a dosage of 1 cc per kg of body weight. The method used for the preparation of renin was essentially that described by Grossman,[9] except that acetone was used as a dehydrating agent and much of the associated protein was removed by isoelectric precipitation.

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[6] Grollman, A., Williams, J. R., Jr., and Harrison, T. R., *J. Am. Med. Assn.*, 1940, **115**, 1169.

[7] Page, I. H., Helmer, O. M., Kohlstaedt, K. G., Fouts, P. J., and Kempf, G. F., *Proc. Cent. Soc. Clin. Res.*, 1940, p. 8.

[8] Johnson, C. A., and Wakerlin, G. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 277.

Blood serums were examined for antirenin before treatment with hog renin and at 2-week intervals subsequently. The technic previously described,[8] consisted essentially in mixing 2 volumes of serum with one volume of renin solution, allowing the mixture to stand at least overnight at 4°C, and assaying the acute pressor effect of the mixture intravenously on the etherized, nephrectomized dog or the brain pithed, nephrectomized cat. The dosage of renin solution employed was 0.5 cc per kg of assay animal. In all instances the serum tested for antirenin was suitably controlled with normotensive dog serum and frequently with serum from untreated hypertensive dogs. Antirenin titers for both hog and dog renins were determined.

At the present writing, one of the dogs has been observed for 7 months and the other 3 for 2 months since hog renin injections were discontinued.

Results. The reductions in the blood pressures of these 4 hypertensive dogs resulting from intramuscular hog renin are illustrated by Figs. 1-4.

The first hypertensive dog showed an average femoral blood pressure of 164 mm of Hg with a maximum of 184 mm and a minimum of 146 mm, during the 3 months preceding treatment. As shown by Fig. 1, the blood pressure of the animal fell more or less steadily throughout the period of hog renin injections until the normal or prehypertensive range was reached in the fourth month of treatment. During the 2 months following renin therapy, the blood pressure dropped somewhat below the original normotensive level. In the succeeding 5 months the pressure slowly rose, so that it has now reached the pretreatment hypertensive range.

The second dog was hypertensive for 9 months prior to renin injections, although only the last 6 months of this period are recorded in Fig. 2. As illustrated, the blood pressure decreased during

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[9] Grossman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 40.

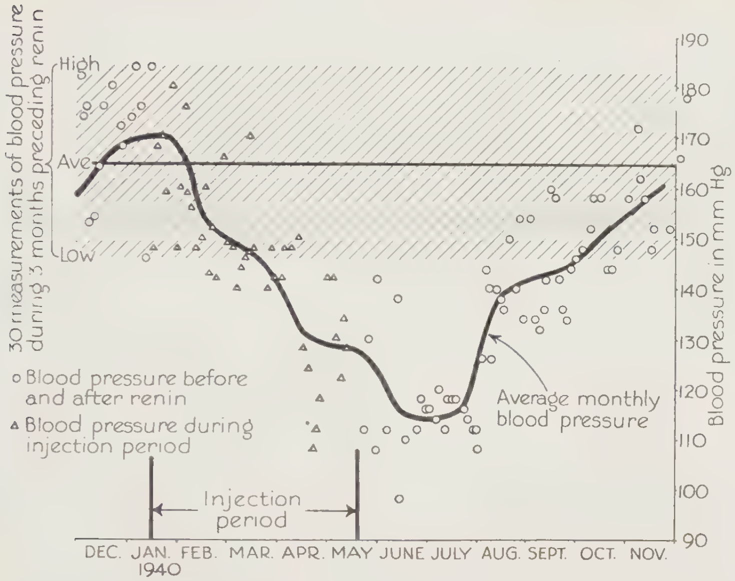

Fig. 1.

The effect of daily injections of hog renin on the blood pressure of renal hypertensive dog No. 1.

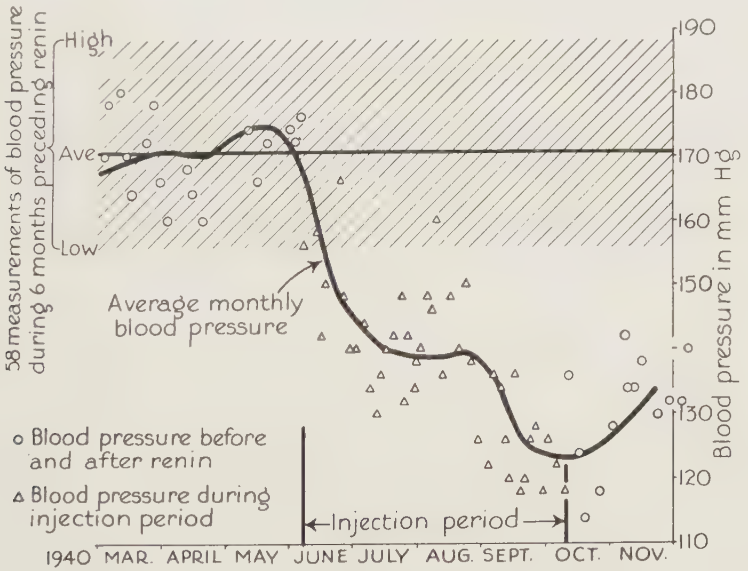

Fig. 2.

The effect of daily injections of hog renin on the blood pressure of renal hypertensive dog No. 2.

the 4 months of treatment until the prehypertensive level of the animal was reached by the end of the third month and a range

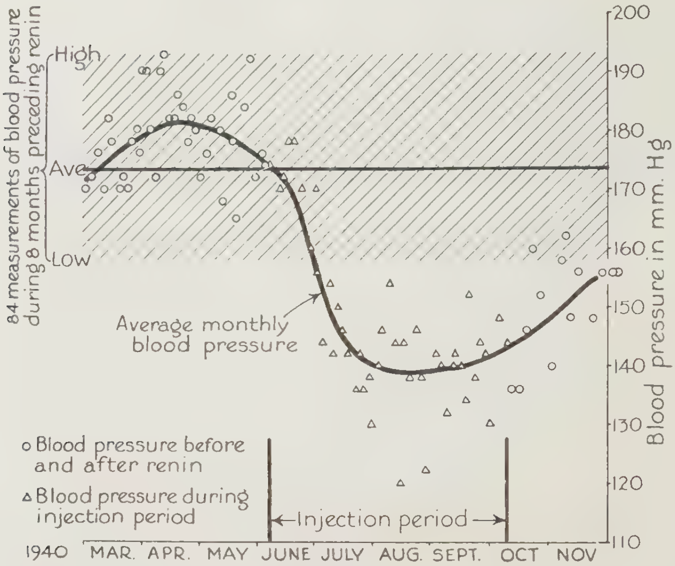

FIG. 3.

The effect of daily injections of hog renin on the blood pressure of renal hypertensive dog No. 3.

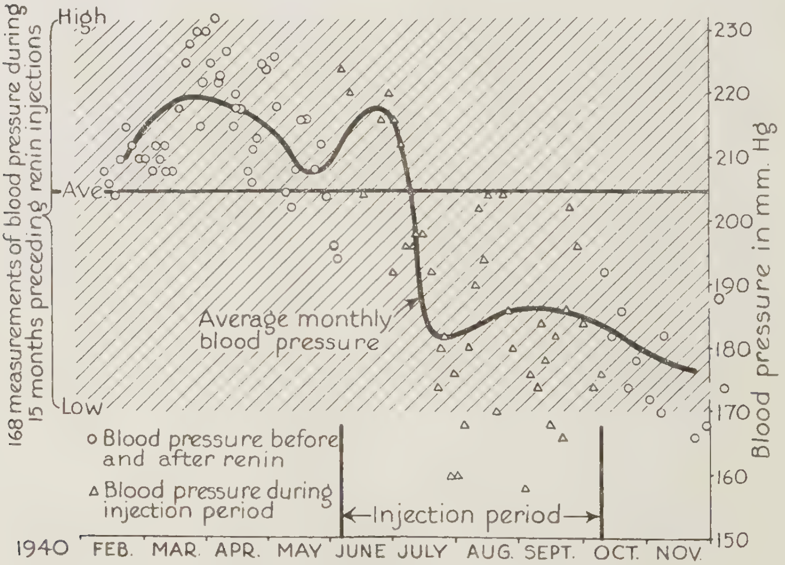

FIG. 4.

The effect of daily injections of hog renin on the blood pressure of renal hypertensive dog No. 4.

slightly below the original normotensive level was attained in the fourth month. During the 2 months following treatment, the pressures have risen to the original normotensive range.

The third dog (Fig. 3) was hypertensive for 17 months prior to renin therapy, although only the last 8 months of this period are recorded. As indicated, the blood pressure fell to the prehypertensive level by the end of the second month of treatment and remained in this range throughout the remainder of the treatment period. During the months which have elapsed since the last renin injection, the blood pressure has shown a gradual rise toward the hypertensive range.

The fourth dog (Fig. 4) was hypertensive for 23 months before hog renin injections were begun, the last 15 months of this period being recorded. The blood pressure of this dog fell to the lower limits of the hypertensive range during the first 2 months of treatment and remained there for the rest of the treatment period. During the 2 months following renin injections the pressures have remained essentially unchanged.

At no time during treatment or subsequently was there any clinical evidence of untoward effects. The appetites of the 4 dogs remained excellent, their weights constant, and their blood urea nitrogens and urinalyses normal throughout the period of observation.

Before hog renin injections, the serums of the dogs showed no neutralizing effect for hog or dog renin. Repeated examinations of the serums of the 20 normotensive and 10 untreated hypertensive dogs used as controls likewise never showed any neutralizing effect for these renins. Antirenin to hog renin became demonstrable by the end of the first month of treatment and with variations in the 4 dogs reached a sufficient titer by the end of the third month to neutralize completely the acute pressor effect of hog renin by the particular assay technic employed. With fluctuations, the antirenin titers for hog renin of the serums of the 4 dogs remained essentially unaltered during the observation periods following hog renin injections (7 months for the first and 2 months each for the other 3 dogs). During hog renin injections and subsequently the neutralizing effects of the serums for dog renin more or less paralleled the antirenin titers for hog renin.

Discussion. This report is obviously preliminary in character. However, the blood pressure reductions obtained in these 4 hypertensive dogs in the absence of any overt evidence of toxic effects are so striking that we have every reason to believe they will be duplicated on a larger series of animals. In our opinion no comparable therapeutic results have thus far been reported in this form of experimental hypertension. In general, there was some correspondence between the magnitude of the fall in blood pressure and the length of the period of hypertension preceding treatment with hog renin. Thus, as indicated in Figs. 1-4, the decrease in blood pressure was greatest in the first dog which had been hypertensive for only 3 months and least in the fourth animal which had been hypertensive for nearly 2 years preceding hog renin injections. Most probably these differences are related principally to the degree of arteriosclerotic change present.

A number of control experiments are obviously necessary: 1. The effect of a similar course of injections of hog renin inactivated by heat must be studied. To date we have treated 2 renal ischemic hypertensive dogs with heat-inactivated hog renin for 4 months without any blood pressure effect, toxic manifestations, or the appearance of antirenin in the serums. These 2 dogs would almost certainly have shown some reduction in blood pressure during the treatment period if active hog renin had been employed. 2. The effect of dog renin and other species of renin must be studied. In this connection, one of us[10] reported that the daily injection of similar doses of dog renin into 2 hypertensive dogs for one and 2½ months respectively had no effect except to elevate slightly the blood pressure of the animals. No antirenin studies were made on these dogs. The second of these animals was the fourth dog (Fig. 4) which received the dog renin injections 18 months prior to hog renin. Consequently, we do not believe that homologous renin will prove effective in reducing the blood pressure of renal hypertensive dogs, although other heterologous renins most probably will.† 3. The effect of highly purified renin preparations, as well as the optimal frequency, dosage, and mode of administration must also be studied. 4. The action of extracts of various tissues from the hog and other species prepared after the manner of renin must be determined.

We have already emphasized the lack of harmful effects in the 4 hypertensive dogs treated with hog renin. The absence of albuminuria is cogent evidence against significant renal damage.[11] However, in view of the deleterious effects of renal extracts in nephrectomized dogs reported by Winternitz, *et al.*,[12] we purpose to examine our treated hypertensive dogs with additional tests of renal, hepatic, and other functions, as well as grossly and microscopically at necropsy. Worthy of note is the fact that, contrary to the suggestion of others,[13] the observed reductions in blood pressure *per se* did not cause toxic manifestations.

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[10] Wakerlin, G. E., and Gaines, W., *Am. J. Physiol.*, 1940, **130**, 568.

† Since the preparation of this report we have injected one hypertensive dog with dog renin for 2½ months without the appearance of antirenin or any effect except slightly to increase the blood pressure.

[11] Brucer, M., and Robinson, S. C., *Am. J. Clin. Path.*, 1940, **10**, 800.

[12] Winternitz, M. C., Mylon, E., Waters, L. L., and Katzenstein, R., *Yale J. Biol. Med.*, 1940, **12**, 623.

Certainly much work remains to be done before the mechanism of this striking reduction in the blood pressure of renal hypertensive dogs by hog renin is clarified. The fact that the hog and dog antirenin titers of the serums of the 4 animals rose simultaneously with the reductions in blood pressure suggests the possibility that antirenin may be responsible. However, the failure of the antirenin titers (especially to dog renin) to fall as the blood pressures of the dogs increased during the months following renin, argues against this. Thus the blood pressure of the first hypertensive dog is now within the original hypertensive range (Fig. 1) and yet the antirenin titer to both hog and dog renin is still such as to give almost complete neutralization of renin pressor effect. Parallel results were obtained when the serums of the 4 treated hypertensive dogs were tested with a crude renin prepared from dog kidney by extraction with physiological salt solution. While the available evidence, therefore, does not favor antirenin as the mechanism responsible for the reductions in blood pressure, this possibility is by no means ruled out. The fact that a heterologous (hog) renin and not the homologous (dog) renin was effective speaks for the probability that the reductions in blood pressure of the 4 hypertensive dogs resulted from some type of immune or antihormone response to renin or less probably to some other heat labile constituent of the renal cortex of the hog. Another, though less likely, possibility is that of a significantly greater concentration of some directly antihypertensive agent in the hog renin solution as compared with the dog renin solution.

Without much question the antihypertensive action of the hog renin injections was not due to the coincidental presence of the antipressor substance under investigation by Harrison and coworkers[6] and by Page and associates.[7] Thus the amounts of kidney equivalent used by them were much larger than those employed by us, the blood pressure increases following cessation of therapy were more prompt in their animals than in ours, and frequently signs of toxicity accompanied their reductions in blood pressure. Moreover,

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[13] Williams, J. R., Jr., Grollman, A., and Harrison, T. R., *Am. J. Physiol.*, 1940, **130**, 496.

Harrison, *et al.*,[14] have shown that their principle inhibits the acute pressor effect of renin and that it is extractable from dog kidney and presumably when so obtained effective in the hypertensive dog.

If the promise of our preliminary findings is substantiated by further work this type of treatment will be studied in essential hypertension in man.

Conclusions. 1. Daily intramuscular injections of hog renin for 4 months produced striking reductions in the blood pressures of renal ischemic hypertensive dogs, whereas heat inactivated hog renin and active dog renin were without effect. 2. No toxic manifestations resulted from renin treatment or from the reductions in blood pressures. 3. The serums of the dogs treated with active hog renin neutralized the acute pressor effect of renin in assay animals. 4. The mechanism of these reductions in blood pressure is not clear. Most probably an immune (antihormone?) response to heterologous hog renin is involved.

11905

Effect of Angiotonin on the Gall Bladder and the Duodenum.

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Angiotonin was first described by Page and Helmer[1] who prepared it by incubating a renin solution with a pseudo-globulin ("activator") fraction of blood serum. Substituting our renin[2] into their process, we too were able to prepare angiotonin. During the period of testing and comparing our product with the original angiotonin some additional pharmacologic actions were observed.

Gall Bladder. Two different series of preparations were used to demonstrate a contraction-stimulating action on the gall bladder. The first group of experiments was performed upon the guinea pig gall bladder (15 tests) according to the *in vitro* technic of Doubilet and Ivy.[3] Strong contractions were observed when relatively large amounts (2.5-4.0 mg) of angiotonin were added to the bath. The second group of studies was performed on 6 dogs which were anesthetized with ether or nembutal. The gall bladder was prepared by the method of Ivy and Oldberg[4] for making direct

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[14] Grollman, A., Williams, J. R., Jr., and Harrison, T. R., *J. Biol. Chem.*, 1940, **134**, 115.

* Florsheim Fellow.

[1] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

[2] McEwen, E. G., Harrison, S. P., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 254.

[3] Doubilet, H., and Ivy, A. C., *Am. J. Physiol.*, 1938, **124**, 379.

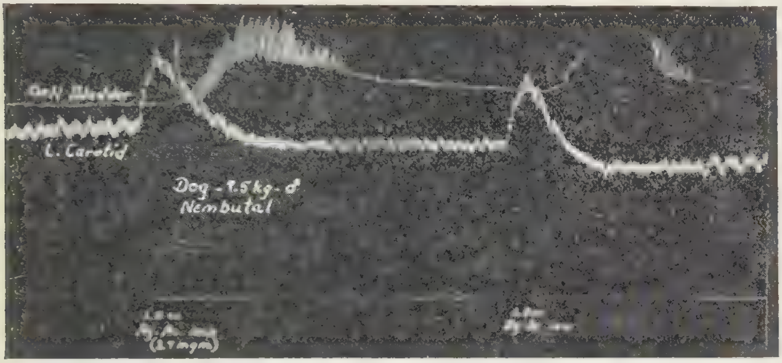

FIG. 1.
Effects of intravenous administration of angiotonin acetate on blood pressure and intracystic pressure of the dog. (In the second response the respiratory excursions have been damped out of the gall bladder curve.)

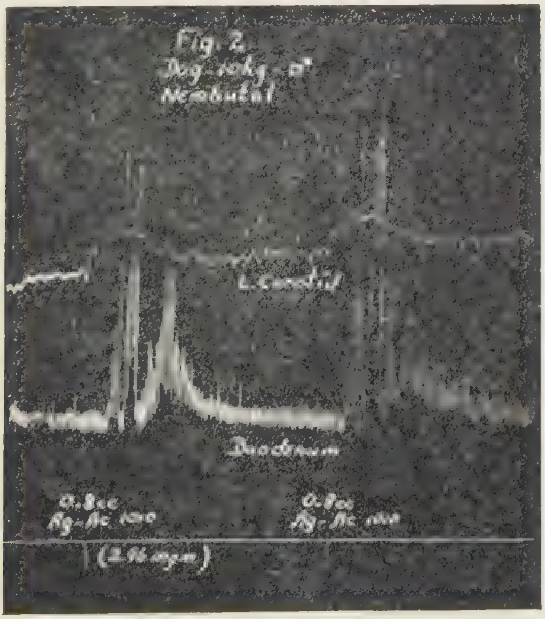

FIG. 2.
Effects of angiotonin acetate on blood pressure and duodenum of the dog.

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[4] Ivy, A. C., and Oldberg, E., *Am. J. Physiol.*, 1928, **86**, 599.

recordings of intra-cystic pressure. It was found in all of 23 tests that the injection of angiotonin into the femoral vein in doses of 0.26-0.64 mg per kilo of body weight caused a prompt contraction of the gall bladder (Fig. 1).

Duodenum. In their original publication Page and Helmer[1] demonstrated that angiotonin would stimulate the tonus and peristaltic motion of isolated strips of rabbit ileum. We have determined whether such an effect could be demonstrated in the intact animal. In 15 dogs the distal segment of the duodenum was exposed and a finger-cot balloon was inserted into the lumen through a stab wound. This was fixed with a purse string suture through the serosa and attached to a water manometer. Tonus and motility were recorded upon a kymograph. It was found that the intravenous administration of angiotonin in doses of 0.15-0.52 mg per kilo of body weight resulted in a marked increase in both tonus and peristaltic activity (Fig. 2).

Control tests using renin and "activator" solutions separately were uniformly ineffective in all of these tests. In addition, it was observed that neither atropine sulphate (0.2 mg per kilo) nor ergotoxine (0.15-0.3 mg per kilo) inhibited the action of angiotonin upon the gall bladder or the duodenal musculature.

Conclusion. Angiotonin stimulates contraction of the isolated gall bladder of the guinea pig and of the *in situ* gall bladder and duodenum of the dog.

11906 P

A New Form of Virus Pneumonitis Occurring Epidemically Among Newborn Infants.

JOHN M. ADAMS. (Introduced by Irvine McQuarrie.)

From the Department of Pediatrics, University of Minnesota, Minneapolis.

A number of reports in the recent literature have called attention to the occurrence of pneumonia in atypical form suggestive of a virus etiology. However, none of them, excepting that of Goodpasture, Auerback, Swanson and Cotter[1] refers to virus pneumonia occurring in infants. The latter authors described pathological changes characteristic of a virus infection of the lungs in 5 babies (ages varying from 2 weeks to 2½ years), dying from pneumonia secondary to measles or whooping cough. These cases occurred sporadically over a period of several years (1931-1938). The most notable feature of the pathology described was the occurrence of intranuclear inclusion bodies in the affected lung tissue.

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[1] Goodpasture, E. W., Auerback, S. H., Swanson, H. S., and Cotter, E. F., *Am. J. Dis. Child.*, 1939, **57**, 997.

The present report deals with a distinctly different and hitherto undescribed form of primary pulmonary disease occurring epidemically among infants in a newborn nursery. Its peculiar symptomatology, epidemiology and pathology indicate the virus nature of its etiology. Characteristic *cytoplasmic* inclusion bodies were found in the epithelial cells of the bronchial tree in all of nine cases terminating fatally. Data pertaining to the clinical course, the epidemiology and the pathology of the disease as observed in the first of 2 small epidemics occurring in Minneapolis and St. Paul are summarized here.*

The disease was characterized by its high degree of contagiousness, by the constancy of the clinical picture from case to case and by the fact that susceptibility was apparently limited to very young infants. While all of the 32 babies exposed to it contracted the disease, not one of their mothers or one of the adult attendants did so. In those cases in which it was possible to ascertain the date of exposure, it was observed that clinical signs of illness appeared after an incubation period of approximately 7 days.

Cough, dyspnea, cyanosis and low-grade fever, often biphasic in character, were the outstanding clinical features. In the average case the intensity of these more obvious signs contrasted sharply with the almost insignificant findings from percussion and auscultation. Roentgen ray examinations of the chest revealed patchy densities indicative of bronchopneumonia.

Nine, or approximately 28% of the 32 infants contracting the disease died. Death appeared to be due to the disease itself in all but one case in which empyema and staphylococcus abscesses of the lung complicated the original infection. The most striking pathological changes found on postmortem examination were those in the lungs. Grossly the affected lungs appeared acutely congested, and scattered, somewhat raised and often hemorrhagic areas stood out on cross section. Thick, ropy mucoid exudate partially filled the bronchial lumen.

Microscopically the exudate was seen to consist largely of sloughing epithelial cells. The bronchi showed areas of necrosis and ulceration with partial collapse of the finer bronchioles and widespread atelectasis. The characteristic cytoplasmic inclusion bodies were found in the epithelial cells of the bronchial tree in all 9 of the fatal cases. In 5 the inclusions were found in the cells within the alveolar spaces. With the hematoxylin and eosin stain the ground substance of the inclusions appeared homogeneous, staining bright red or acidophilic. The diameter of the bodies varied from 3 to 6 microns. A clear halo was noted about the body in many places, and small vacuoles were seen within the ground substance of several inclusions.

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* Biological studies of the virus, now being carried out in collaboration with Dr. R. G. Green and Dr. C. A. Evans of the Department of Bacteriology, will be published at a later date.

The serum of ferrets, which were inoculated with fresh frozen lung specimens, failed to show any antibodies capable of neutralizing influenza virus.[†]

Repeated examinations of the bronchial exudate showed type specific pneumococci in but one of the fatal cases (type XXVII) and in but 6 of the cases surviving (4 of type XXIII and 2 of type XXVII).

Summary. A new form of primary, acute pneumonitis of infants is described. The disease is characterized by its predilection for young infants, its epidemic nature or high degree of contagiousness, the constancy of its symptomatology from case to case and its pathology, including the occurrence of typical cytoplasmic inclusion bodies in the epithelial cells of the bronchial tree.

11907

Anesthetic Effect of Steroid Hormones.*

HANS SELYE.

From the Department of Anatomy, McGill University, Montreal, Canada.

In the course of our experiments on the physiology of steroid hormones, we were surprised to note how difficult it is to produce acute overdosage phenomena even if enormous doses are given. Thus even a single subcutaneous injection of 100 mg of testosterone, progesterone or desoxycorticosterone acetate (D.C.A.) given in 4 ml of peanut oil to rats weighing 100 g caused no detectable ill effects. It was felt that this might be due to the slow rate of absorption of oily solutions from the subcutaneous tissue. As most of these compounds are practically insoluble in water and hence cannot be given intravenously, we recently administered a series of steroid hormones in oily solution by the intraperitoneal route in the hope that from this location, absorption may be especially rapid and that we may thus learn more about the pharmacological effects of acute overdosage with steroids.

[†] The author is indebted to Dr. Thomas Francis, Jr., for carrying out this test.

[*] The expenses of this investigation were defrayed through a grant in aid received from the Schering Corporation of Bloomfield, New Jersey. The author is also indebted to Dr. A. D. Odell of Charles E. Frosst and Company for the androsterone, to Dr. Stanley Cook of Ayerst, McKenna and Harrison, Ltd., for the pregnanediol, and to Drs. G. Stragnell and E. Schwenk of the Schering Corporation for all the other steroids used in this investigation.

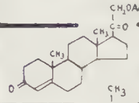
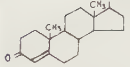
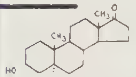
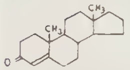
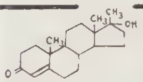
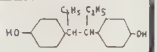
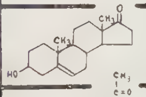
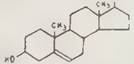
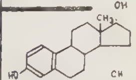
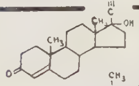
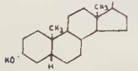
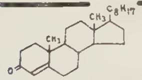
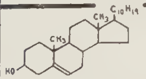
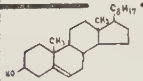
For our first experiment, we divided 30 male and 30 female albino rats weighing 90-135 g (average: 107 g) into 5 groups of 6 males and 6 females each. The first of these groups was treated with D.C.A., the second with progesterone, the third with testosterone, the fourth with α-estradiol and the fifth with cholesterol. Each animal received a single intraperitoneal injection of 35 mg of the compound in 1.5 ml of peanut oil. Fifteen minutes after the injection, all the females in the D.C.A. and progesterone groups were deeply anesthetized but only one male of the former and 2 of the latter group showed a comparable degree of anesthesia while the remaining males were either quite normal or only very slightly anesthetized. The females of the testosterone group were also deeply anesthetized but more than one hour was required for this effect to take place. However, here again, the males—though not larger in size than the females—were not nearly as much affected as the latter. The animals receiving estradiol or cholesterol showed no signs of anesthesia and survived the injection indefinitely. On the other hand, all females in the progesterone and D.C.A. groups died within 6 hours after the injection as well as one of the males in each of these 2 groups. Among the testosterone-injected rats, only one female died. The surviving animals of all groups awakened within a few hours and showed no ill effects after the anesthesia. This experiment indicated that progesterone and D.C.A. may both lead to acute overdosage symptoms if administered by the intraperitoneal route and that their anesthetic effect as well as their toxic action is greater in females than in males. These observations were confirmed by many subsequent experiments on male and female rats and mice of different sizes. Although space does not permit the full description of all our observations, we should like to emphasize that the individual sensitivity of the animals shows wide variations but if these active steroids are administered in suitable doses they cause a quiet deep anesthesia which is not preceded by any obvious sign of excitation and from which the animals recover without ill effects. This anesthesia is so deep that we often performed prolonged abdominal operations in rats anesthetized with D.C.A. or progesterone. At the height of the anesthesia, there is some peripheral vasodilatation which leads to hyperemia of the paws and ears and a slight decrease in body temperature. If lethal doses are administered, death is apparently due to anesthesia of the respiratory centers since outside of occasional small extravasations in the lungs, no obvious pathological changes could be detected and the heart continued to beat long after respiration stopped. The fact that it is really the increased rate of absorption which is responsible for this anesthetic action received support from the observation that general anesthesia may also be produced by the intravenous administration of D.C.A. in propylene glycol solution.

In order to obtain some information concerning the correlation between chemical structure and anesthetic effect, a series of compounds have been tested. In some cases part of the compound had to be given in a fine crystalline suspension rather than in solution. Most of them, however, could be kept dissolved in the concentration used by heating the solutions and then cooling them down to body temperature just before the injection. Each compound was tested on a group of 8 female albino rats weighing 79-130 g (average: 105 g). Five mg of each compound were injected in 0.25 ml of peanut oil every 30 minutes until 50% of the animals in a group were completely anesthetized. Table I compares the chemical structure of the compounds used with their anesthetic action. Some of these compounds could not be obtained in absolutely pure form and therefore we determined the melting points of all our samples to indicate the degree of their purity. No effort has been made as yet to determine the safety margin between the full anesthetic and the lethal dose but the fact that many of our animals died should not lead to the conclusion that this safety margin is small since for the sake of uniformity, we reinjected all animals of the groups in which more than 50% were still awake, including those which were already deeply anesthetized. It should also be emphasized that testosterone, though distinctly anesthetic, appears to have a very delayed action so that the full effect of the preceding dose was not yet in evidence after 30 minutes when the next dose was to be injected. Unless a lower dose caused deep anesthesia in 50% of the animals, injections were continued at the rate of 5 mg every half hour until a total dose of 50 mg was reached.

This table in which the compounds are arranged as far as possible

TABLE I.

ANESTHETIC EFFECT OF STEROIDS

COMPOUND	FORMULA	M.P. OF SAMPLE USED	DOSE REQUIRED TO PRODUCE DEEP ANESTHESIA IN 50% OF ANIMALS	DEATHS OUT OF 8 RATS	REMARKS
DESOXYCORTICOSTERONE ACETATE		149-150°	5 MG.		ANESTHESIA NOT PRECEDED BY EXCITATION OR CONVULSIONS.
PROGESTERONE		128°	15 MG.	1	ANESTHESIA NOT PRECEDED BY EXCITATION OR CONVULSION.
ANDROSTERONE		177-178°	50 MG.	2	SLOWLY DEVELOPPING BUT VERY PROLONGED ANESTHESIA RARELY PRECEDED BY CONVULSIONS.
TESTOSTERONE		153°	50 MG.	4	SLOWLY DEVELOPPING BUT VERY PROLONGED ANESTHESIA IN SOME CASES PRECEDED BY CONVULSIONS.
METHYLTESTOSTERONE		160°	50 MG.		SOMEWHAT LESS POTENT THAN TESTOSTERONE BUT HAS ACTIVITY OF SIMILAR TYPE.
DIETHYLSTILBESTROL		166°	———	1	50 MG. CAUSE SLIGHT ANESTHESIA IN SOME ANIMALS. LARGER DOSES ELICIT DEEP ANESTHESIA WITHOUT CONVULSIONS.
Δ5-DEHYDRO-ISO-ANDROSTERONE		137°	———	3	50 MG. CAUSE ONLY SLIGHT ANESTHESIA PRECEDED BY INTENSE CONVULSIONS.
Δ5-PREGNENE-3(β)-OL-20-ONE		183°	———	—	IN 50 MG. DOSE ACTIVITY VERY SLIGHT.
α-ESTRADIOL		176°	———	—	IN 50 MG. DOSE ACTIVITY QUESTIONABLE.
ETHINYLTESTOSTERONE		265-268°	———	—	INACTIVE UP TO 50 MG. BUT THIS MAY BE DUE TO ITS VERY LOW SOLUBILITY IN OIL.
PREGNANE-3(α),20(α)-DIOL		237-238°	———	—	INACTIVE UP TO 50 MG. BUT THIS MAY BE DUE TO ITS VERY LOW SOLUBILITY IN OIL.
Δ4-CHOLESTENONE		80.5°	———	—	INACTIVE UP TO 50 MG.
STIGMASTEROL		168-169°	———	—	INACTIVE UP TO 50 MG.
CHOLESTEROL		148°	———	—	INACTIVE UP TO 50 MG.

in decreasing order of activity shows no evident correlation between chemical structure and activity. However, only hormonally active steroids proved to have an anesthetic effect even though the activity was not limited to compounds with any one specific type of hormonal action.

Another experiment was planned to determine the site at which detoxification of acutely administered steroids takes place. Three groups of rats were used. Each of these consisted of 12 females weighing 138-220 g (average: 190 g). The first group was partially hepatectomized according to the method of Waelsch and Selye[1] while the second group was bilaterally nephrectomized. On the day after the operation, all these rats as well as the third group of intact controls received 2 intraperitoneal injections of 5 mg of D.C.A. in 0.25 ml of peanut oil, the second injection being given 30 minutes after the first. Just before the second injection, 4 of the hepatectomized rats were in deep anesthesia, 2 were still unaffected while the rest were but slightly anesthetized. At this time, none of the nephrectomized or intact rats showed any signs of anesthesia. Two hours after the second injection 2 of the hepatectomized animals died, the rest were in deep anesthesia. Among the nephrectomized animals, 4 were in deep anesthesia but the rest were entirely unaffected. Subsequently, the hepatectomized animals remained in anesthesia until they died while the nephrectomized animals recovered completely from the anesthesia before they eventually died from uremia. The intact rats never showed any signs of anesthesia from this dose of D.C.A. which is below the anesthetic level for normal animals of this size. It is evident that hepatectomy increases sensitivity to the anesthetic effect of D.C.A. This probably indicates that the liver plays an important rôle in the detoxification of this compound since Waelsch and Selye[1] showed that anesthetics—such as tribromethanol (avertin)—which are detoxified in the liver keep partially hepatectomized animals asleep for an unusually long period while the anesthesia caused by magnesium salts—which are not detoxified by the liver—remains uninfluenced by this intervention. Whether the slight increase in D.C.A. sensitivity due to nephrectomy should be interpreted as showing that this organ also plays a rôle in the detoxification of steroids is open to question since nephrectomy damages the animals and may thus sensitize them in a non-specific manner.

The fact that absorption of these water-insoluble, oil-soluble compounds may greatly be accelerated by administering them to surface membranes is perhaps of more than purely academic interest especially since a similar improvement in absorption rate may perhaps be achieved in man by applying such compounds to mucous membranes.

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[1] Waelsch, H., und Selye, H., *Naunyn-Schmidebergs Arch.*, 1931, **161**, 115.

Summary and Conclusions. Various steroid hormones, especially D.C.A. and progesterone produce deep anesthesia in rats and mice if injected into the peritoneum whence they can be rapidly absorbed. After recovery from the anesthesia such animals show no ill effects. Partially hepatectomized rats are much more sensitive to the anesthetic effect of the steroids than intact controls. This probably indicates that the liver plays an important rôle in the detoxification of these compounds. Males are less sensitive than females of the same size.

11908

Availability of Sodium Pyruvate for Human Brain Oxidations.*

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We have previously described a method for the study of the availability of various substrates for human brain metabolism *in vivo.*[1] In the absence of glucose during therapeutic insulin shock it has been shown that the metabolism of the brain is diminished. This is indicated by an extremely low oxygen uptake of the brain[2, 3] in association with characteristic changes of the cortical brain potentials[4] and the onset of clinical coma. Intravenous administration of glucose rouses the patient and restores the normal arterio-venous oxygen difference.[5] In previous publications we have reported that as little as 4 g of glucose administered intravenously invariably roused the patients from coma and approximately doubled the oxygen uptake of the brain.[6] Ethyl alcohol had no effect on the clinical state or the oxygen uptake of the brain,[7] while racemic sodium lactate resulted in only a slight increase in the oxygen uptake of the brain, and had no effect on the comatose state of the patients.[8] In this report we are presenting evidence on the availability of sodium pyruvate for brain oxidations.

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* Aided by a grant from the Havelock Ellis Fund for Psychiatric Research.

[1] Wortis, J., and Goldfarb, W., *Science,* 1940, **91**, 270.

[2] Damashek, W., Myerson, A., and Stephenson, C., *Arch. Neur. and Psychiat.,* 1935, **33**, 1.

[3] Himwich, H. E., Bowman, K. M., Wortis, J., and Fazekas, J. F., *J. Nerv. and Ment. Dis.,* 1939, **89**, 273.

[4] Hoagland, H., Cameron, D. E., and Rubin, M. A., *Am. J. Psychiat.,* 1937, **94**, 183.

[5] Himwich, H. E., Frostig, J. P., Fazekas, J. F., and Hadidian, Z., *Am. J. Psychiat.,* 1939, **96**, 371.

Experimental. The method has been previously described.[6] The cerebral metabolism was estimated from the arterio-venous differences of O_2, CO_2, and glucose. Duplicate analyses of blood gas determinations checked within 0.2 vol. %. The velocity of circulation in the peripheral vascular system was estimated with the method of Robb and Weiss.[9] In 9 experiments the metabolism of the brain was estimated after the patients were in deep hypoglycemic coma, and again 15 minutes after the administration of various amounts of sodium pyruvate. The data from these experiments are presented in Table I. Patients 1 and 6 roused slightly after administration of the pyruvate, but soon lapsed into coma again. The remaining patients did not rouse. The doses varied from 4 to 8 g of pyruvate, and are indicated in the table. In 4 additional patients 15 g of sodium pyruvate were administered, and in none of these cases did the patient rouse from the comatose state. The average oxygen uptake during coma was 2.9 volumes %, and after the pyruvate was 4.08 volumes %. Statistical analyses of these averages reveal that the difference was barely significant. It is interesting to note that this increase closely

TABLE I.
Effect of Sodium Pyruvate Administered Intravenously on Cerebral Metabolism of Schizophrenic Patients in Insulin Coma.

Exp. No.	Dose of pyruvate, g	Volume % oxygen			Circ. time, sec.
		art.	ven.	diff.	
1	4.0	19.5	15.1	4.4	15
2	6.7	19.9	15.0	4.9	
3	8.0	20.9	16.9	4.0	12
4	8.0	19.1	16.0	3.1	10
5	8.0	23.2	21.6	1.6	12
6	8.0	20.0	14.6	5.4	11
7	8.0	19.5	15.2	4.3	10
8	8.0	19.6	16.1	3.5	9
9	8.0	20.8	15.3	5.5	10
	Avg	20.26	16.20	4.08 ± 1.10	11.1
Avg of 60 cases in insulin coma		19.6	16.7	2.90 ± 1.49	11.8
	Difference ± St. Deviation = 1.18 ± 0.418.				

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[6] Wortis, J., and Goldfarb, W., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 382.

[7] Goldfarb, W., and Wortis, J., *Quart. J. Studies in Alcohol*, 1940, **1**, 268.

[8] Wortis, J., and Goldfarb, W., *Am. J. Physiol.*, 1940, **129**, 502.

[9] Robb, G. P., and Weiss, S., *Am. Heart J.*, 1932-3, **8**, 650.

approximates the increase previously observed after lactate administration.

The estimation of brain metabolism in these experiments was based on the differences between the concentrations of various substances in the arterial and venous blood. This method is subject to error if the blood flow varies, and other investigators have suggested that changes in blood flow can account for the changes observed. In collaboration with Himwich, *et al.*,[10] we have confirmed the observations of Loman and Myerson[11] that in the absence of convulsions there is no appreciable change of the brain blood flow during insulin coma. During the course of this research we also observed that the administration of glucose, lactate and pyruvate did not affect the blood flow of the brain of patients during insulin coma. It therefore appears probable that the changes observed in the arterio-venous oxygen difference of the brain indicates that there is a corresponding increase of the brain metabolism after the administration of sodium pyruvate, but this increase is not sufficient to rouse patients from coma. These results are not in agreement with the effects of pyruvate on the metabolism of isolated brain tissue in the Warburg apparatus. The possibility remains, however, that the oxidations *in vitro* occur at a diminished rate[12] and may be maintained by the oxidation of pyruvate, while the oxidation of pyruvate *in vivo* is too slow to supply sufficient energy for brain metabolism during insulin hypoglycemia.

Summary and Conclusions. The availability of sodium pyruvate for brain oxidations in hypoglycemic insulin coma was studied in human patients. In 2 of 14 experiments with various amounts of pyruvate the patients roused slightly from coma, and in the remaining 12 there was no change observed in the clinical state. In 9 patients the oxygen uptake of the brain was observed, and there was a small, though statistically significant, rise. These results are in striking contrast to those observed with glucose, and indicate that the oxidation of pyruvate by the brain is not sufficient to adequately support cerebral functions.

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[10] Himwich, H. E., Bowman, K. M., Daly, C., Fazekas, J. F., Wortis, J., and Goldfarb, W., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 468.

[11] Loman, J., and Myerson, A., *Am. J. Psychiat.*, 1936, **92**, 791.

[12] Gerard, R. W., *Ann. Rev. Biochem.*, 1937, **6**, 419.

11909

Continued Administration of Sulfathiazole on Renal and Hepatic Function in the Dog.

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Clinical experience with sulfapyridine has indicated that haematuria, associated with urolithiasis, occurs occasionally from the administration of this drug.[1, 2, 3] Molitor, Antopol and Robinson[4, 5] succeeded in demonstrating the etiological relationship between the drug and the pathological lesion under experimental laboratory conditions. In the course of our early investigations on the pharmacological properties of sulfathiazole it became evident that this compound, like sulfapyridine, could give rise to experimental urolithiasis and, as a direct result, produce varying degrees of physical irritation in the renal tracts of animals maintained on large continued doses. However, no data on the effects of sulfathiazole on kidney function are available, nor has any study of its effects on the acid-base balance, similar to that of Marshall, *et al.*,[6] on sulfanilamide, been made.

The primary purpose of this investigation has been to ascertain whether or not the continued administration of large doses of sulfathiazole would result in any significant impairment of renal function; secondarily, to determine whether or not there is any alteration of the alkali reserve during such a regime. Finally, in the light of certain of our experimental findings, it became necessary to duplicate the experimental conditions in order to study liver function.

Experimental Methods. The subjects were 3 normal, adult, female dogs trained for the necessary handling. During a preliminary control period frequent determinations were made of accepted criteria for the efficiency of kidney and liver function. The dogs then received 150 mg per kg per day of sulfathiazole for 4 days,

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[1] Snapper, I., Liu, S. H., and Chang, H. L., *Nederl. Tijschr. v. Geneesk.*, 1939, **23**, 4501.

[2] Backhouse, T. C., *Lancet*, 1939, **2**, 736.

[3] Gross, P., Cooper, F. B., and Lewis, M. L., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 448.

[4] Molitor, H., and Robinson, H., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 409.

[5] Antopol, W., and Robinson, H., Proc. Soc. Exp. Biol and Med., 1939, **40**, 428.

[6] Marshall, E. K., Cutting, W. C., and Emerson, K., *J. A. M. A.*, 1938, **110**, 252.

followed by 250 mg per kg per day for 10 days.* Observations were continued for a period of several days after withdrawal of the drug in order to reëstablish normal conditions.

The drug was administered daily at 8:30 A.M., 4:30 P.M., and 11:30 P.M. in capsule form; we had previously observed that a continued blood concentration at a reasonably constant level might be maintained by dividing the daily dose in 3 equal parts and administering at the above time intervals. Exceptionally, sharp falls in the blood concentration were observed, due in all instances to repeated emesis which occasionally followed the administration of the drug. It should be pointed out at this time that the administration of doses of sulfathiazole of this order to dogs is associated with severe anorexia and it was necessary to change from the routine laboratory diet to more acceptable foods in order to avoid complicating the picture by a superimposed inanition.

Daily blood and urine (catheterized) samples were taken 3 hours and 4 hours respectively after the morning dose.

As indices of kidney function we chose the phenolsulphonphthalein excretion rate, the blood urea level, and the urea clearance, the last of which was determined by the method of Summerville, Hanzal and Goldblatt.[7] The urea clearance values reported here are the "standard clearances per square meter" calculated in the manner described by those authors. The other values described above were determined by the standard clinical methods.

Sulfathiazole concentrations in the blood and urine were determined by a modification of Marshall's method. The CO_2 and O_2 content of the blood and the O_2 capacity were determined by Van Slyke's manometric methods. The efficiency of liver function was studied by the bromsulphonphthalein excretion method.

Results. The results are presented in diagrammatic form in Fig. 1.

The blood sulfathiazole level produced by 150 mg per kg per day was approximately 8 mg per 100 cc. When the dose was increased to 250 mg per kg per day, the blood level increased to about 16 mg per 100 cc. The blood concentration persisted for about 24 hours after the cessation of medication and then rapidly fell to zero during the succeeding 24-48 hours.

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* These dose levels approximate 3 and 5 times the average human therapeutic dose administered over 24 hours, and give rise to blood concentrations of from 15-20 mg per 100 cc. In our previous work on this subject we found that the maintenance of blood concentrations of this order for such a period of time was associated with urolith formation.

[7] Summerville, W. W., Hanzal, R. F., and Goldblatt, H., *Am. J. Physiol.*, 1932, **102**, 1.

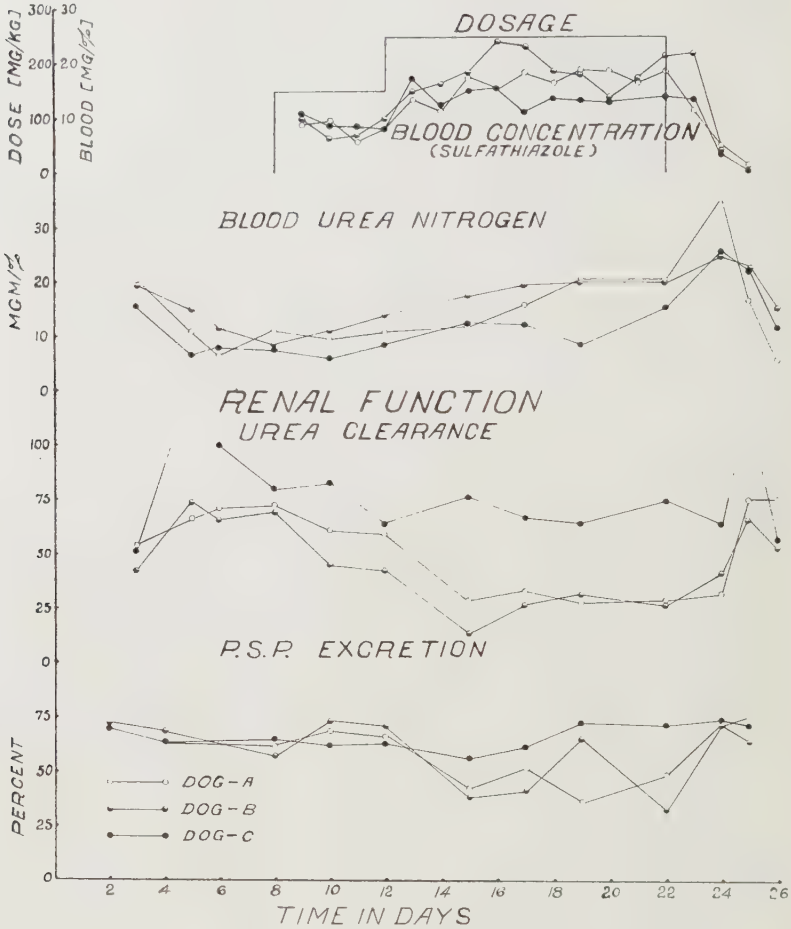

Fig. 1.

In the period prior to medication the blood urea nitrogen averaged about 10 mg per 100 cc. This value began to rise slowly but steadily throughout the entire period of medication, finally reaching a level of about 18 mg per 100 cc at the time the drug was withdrawn. However, all observations made during this period were within the established normal values. A sudden rapid rise occurred in the post-medication period to a mean value of about 30 mg per 100 cc after which there was an extremely rapid fall to the pre-medication level.

With respect to urea clearance, 2 cases must be distinguished. Prior to medication, Dogs A and B showed a urea clearance of about 70. With the beginning of medication they immediately began to fall off until they reached a new level of about 30. With the cessation of medication, the urea clearances of both dogs rapidly returned to their normal levels. On the other hand, Dog C, which was more erratic and occasionally showed extremely high levels, had apparently reached a static urea clearance of about 85 and fell off only very slightly during the medication period. This animal appeared to tolerate sulfathiazole better than the other 2 in all respects. This difference in behavior was not the result of faulty absorption of the drug from the gastrointestinal tract, for the blood sulfathiazole concentration in this animal was of the same order as that of the others.

All 3 animals showed P.S.P. excretion values in excess of 60% within 2 hours during the premedication period. During medication the excretion values of Dogs A and B fell to a level as low as 40%, but returned to a normal level rapidly after medication ceased. The P.S.P. excretion for Dog C was analogous to the urea clearance for this animal in that normal values were maintained throughout.

During the course of medication, frequent urinalyses were made. Both occult and frank blood and traces of albumin appeared coincidentally in the later stages of medication in all dogs. Glycosuria was never observed. As soon as the dose level reached 250 mg per kg per day, large quantities of crystalline sulfathiazole appeared in the urine. The terminal portions of the catheter specimens contained dense clouds of these crystals. Such crystals often appeared as accretions about the urethral meatus. Cystoscopic examination was carried out on the twelfth day of medication and revealed an acute inflammatory reaction of the vesical mucosa of sufficient intensity to obscure all landmarks. In spite of this, the animals appeared to be in no discomfort and the cystitis and urethritis was never sufficiently severe to interfere with the passage of the catheter.

Daily determinations of O_2 capacity, O_2 and CO_2 content failed to reveal any variations beyond the expected daily fluctuations, indicating that no significant quantity of abnormal haemoglobin pigments is formed as a result of the administration of this drug and that there is no primary CO_2 deficit of the type associated with the administration of sulfanilamide.[8] It should be noted, however, that the dose of sulfathiazole administered in this experiment is less than half the amount of sulfanilamide required to produce acidosis.

Periodic examination of the blood picture throughout the course of the experiment failed to reveal any significant changes.

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[8] McChesney, E. W., Marshall, I. H., and Sprague, K. D., *J. Lab. and Clin. Med.*, in press.

Discussion. An examination of the data shows that the administration of doses of sulfathiazole of this order is capable of producing moderate interference with the efficiency of renal function. Such changes are reversible since there is an immediate resumption of normal renal efficiency on withdrawal of the drug. It seems likely that impairment of renal efficiency is primarily due to physical blocking of the renal tract resulting from the accumulation of this poorly soluble drug, rather than to any toxic effect of the drug on the renal tissue. If the latter were the case, one would not expect to find such prompt recovery on discontinuance of medication.

An interesting phenomenon brought out in this study is the sudden sharp rise in the urea nitrogen content of the blood associated with a simultaneous rise in the urea clearance level soon after cessation of medication. While we do not entirely understand this phenomenon, there is a strong suggestion that the animals were in a state of positive nitrogen balance during the phase of maintained sulfathiazole blood concentration, and this increase was associated with a flushing out of the retained nitrogen. It occurred to us that some impairment of hepatic function resulting in the storage of amino nitrogen might have caused this phenomenon; accordingly, we repeated the experimental conditions on the same animals and observed the efficiency of liver function by means of the bromsulphonphthalein excretion test. Repeated tests indicated that no significant retention of the dye occurred under the experimental conditions established. While it must be recognized that this is not a crucial test of the deamination function, it does indicate that liver function was not impaired. Obviously, this phenomenon deserves further study.

Conclusions. 1. The continued administration of sulfathiazole to dogs over a period of 10 days in doses of 250 mg per kg per day produces slight impairment of renal function as evidenced by elevation of the blood urea nitrogen level, and diminution in the urea clearance and P.S.P. excretion. 2. This impairment of function is reversible and normal values are reëstablished within 48 hours after the withdrawal of the drug. 3. Doses of this order have no demonstrable effect on O_2 capacity, O_2 and CO_2 content. 4. Doses of this order have no demonstrable effect on liver function as indicated by the rate of excretion of bromsulphonphthalein.

11910 P

Reabsorption of Certain Amino Acids and Derivatives by the Kidney Tubules.

J. R. Doty. (Introduced by Richard Ashman.)

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Evidence has been presented previously showing a marked variation in the extent of the excretion of different amino acids after their intravenous administration.[1] This study was designed to measure the rates of tubular reabsorption of l-tyrosine, N-acetyl-l-tyrosine, N-methyl-l-tyrosine and l-histidine. The results were obtained by using sodium ferrocyanide to measure the rate of glomerular filtration during the period of study of each substance. The procedure is an adaptation of one elaborated by Van Slyke, Hiller and Miller.[2]

Dogs received an intraperitoneal injection of 200 cc of a solution containing a suitable amount of sodium ferrocyanide together with the compound being studied. After a lapse of 15 minutes the bladder was emptied by catheterization and washed with distilled water. The urine was collected for a period of approximately one hour. Tyrosine and its derivatives were determined by the Folin-Ciocalteu phenol reagent; histidine by the Jorpes modification[3] of the Koessler-Hanke method.[4] A sample of blood was obtained about 15 minutes after the beginning and again 15 minutes before the end of the experimental period. The average of the values for the 2 samples of blood was used in each calculation. Volume of glomerular filtrate was obtained by dividing 100 x mg of ferrocyanide excreted by the average mg % of ferrocyanide in the plasma. By using this volume of glomerular filtrate and the plasma concentration of amino acid it was possible to calculate the amount of amino acid in the glomerular filtrate. Knowing the amount of amino acid excreted one obtained by difference the amount of the amino acid reabsorbed by the tubules.

From Table I it appears that only a small fraction of the acetyl derivative is reabsorbed whereas approximately two-thirds of the methyl derivative is taken up under similar conditions. It is difficult to raise the plasma concentration of tyrosine to a level com-

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[1] Eaton, A. G., and Doty, J. R., *J. Nutrition,* 1941, **21**, 25.

[2] Van Slyke, D. D., Hiller, A., and Miller, B. F., *Am. J. Physiol.,* 1935, **113**, 611.

[3] Jorpes, E., *Biochem. J.,* 1932, **26**, 1507.

[4] Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.,* 1919, **39**, 497.

TABLE I.
Tubular Reabsorption of Amino Acids and Derivatives.

			Amt amino acid				
Dog No.	Compound administered	Vol. of glomerular filtrate, × 100 cc/hr	In plasma, mg%	In glomerular filtrate, mg/hr	In urine, mg/hr	Reabsorbed by kidney tubules	
						mg/hr	%
1	N-acetyl-l-tyrosine	20.1	72.0	1447	1145	302	21
1	” ”	22.5	51.0	1147	1010	137	12
1	” ”	15.3	108.0	1652	1646	6	0.4
2	l-tyrosine	29.0	15.5	450	15	435	97
1	”	17.8	12.0	214	8	206	96
1	N-methyl-l-tyrosine	22.7	44.0	999	355	644	64
1	l-histidine	14.8	41.0	607	6	601	99

parable with that obtained with the other compounds but under the conditions of the experiments the reabsorption of this amino acid is practically complete. All but a trace of histidine is reabsorbed even when the plasma level of this substance is rather high. It is obvious that these rates of reabsorption must be higher than indicated if there is a pronounced "tubular excretion" of these compounds. It seems unlikely, however, that "tubular excretion" could have a significant effect at the higher plasma levels.

It is interesting to note that N-acetyl-l-tyrosine is almost quantitatively excreted[5] whereas tyrosine[6] and histidine[1] are quite rapidly metabolized by the dog. Separate analyses of the filtrates of whole blood and of plasma show that very little of the compounds enters the blood cells during the course of the experiments.

The results presented here seem to offer a promising mode of attack upon the fundamental problem of the mechanism of selective absorption of amino acids by the kidney tubules. Furthermore, such data extended to other amino acids would seem to be essential for a knowledge of the limit to which the plasma concentrations of amino acids could economically be raised during parenteral administration of these substances.

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[5] Doty, J. R., unpublished data.

[6] King, F. B., and Rapport, D., *Am. J. Physiol.*, 1933, **103**, 288.

11911

Use of Para-Aminobenzoic Acid to Inhibit Sulfonamide Action in Bactericidal Tests.

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(Introduced by W. T. Salter.)

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The use of sulfonamide drugs in the treatment of bacterial infections has raised new problems in the study of immunological changes occurring during the course of disease. Blood samples obtained from patients under treatment with sulfonamides frequently contain concentrations of these drugs which in themselves are bactericidal, thus masking immune reactions. Addition of appropriate amounts of *p*-aminobenzoic acid to such blood samples inhibits sulfonamide action.[1] Although antibody activity, in the case of the pneumococcus, may be differentiated from drug action, if bactericidal tests are terminated at 3 hours,[2] this procedure is not always desirable, nor is it feasible with certain other bacteria. It seemed necessary to determine whether or not *p*-aminobenzoic acid inhibits the sulfonamide effect without interfering with the bactericidal action of antibody. The following studies were therefore carried out.

Bactericidal tests were done on fresh defibrinated blood, using stock strains of pneumococci as in previous studies from this laboratory.[3] Sulfathiazole and *p*-aminobenzoic acid were added to the blood separately and in combination. In Experiment I, blood was obtained from a normal subject with a high titer of "natural" pneumococcidal antibodies. In Experiment II the subject was convalescent from Type I lobar pneumonia; in Experiment III a subject whose blood lacked bactericidal action for Type V pneumococcus was chosen and the appropriate antibody added *in vitro*.

The results are shown in Table I. Neither the natural bactericidal action of defibrinated blood nor the bactericidal action associated with recovery from pneumococcus infection was interfered with by the addition of *p*-aminobenzoic acid. Blood which had no

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[1] Strauss, E., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, in press.

[2] Finland, M., Lowell, F. C., and Spring, W. C., Jr., *New Eng. J. Med.*, 1940, **222**, 739.

[3] Spring, W. C., Jr., Lowell, F. C., and Finland, M., *J. Clin. Invest.*, 1940, **19**, 163.

TABLE I.

Effect of Antibody, Sulfathiazole and Para-aminobenzoic Acid, Alone or in Combination, on Pneumococcidal Action of Fresh Defibrinated Human Blood.

Exp. No.	Material added to blood	Concentration, mg%	Growth inhibition		Pneumococcidal action at 48 hr		Remarks
			24 h.	48 h.	Inoculum	Growth	
I	Control		10^6	10^5	10^5	0	Normal subject with "natural" bactericidal antibody. Stock Type I pneumococcus used.
	PABA	1	10^6	10^5	10^5	0	
	ST	10	10^6	10^6	10^6	1	
	PABA ST	1 10	16^6	10^5	10^5	0	
II	Control		10^7	10^7	10^7	0	Convalescent from Type I pneumococcus pneumonia; blood taken 7 days after crisis and 2 days after cessation of sulfonamide therapy. No serum treatment given. Stock Type I pneumococcus used.
	PABA	1	10^6	10^6	10^6	0	
	ST	10	10^7	10^7	10^7	0	
	PABA ST	1 10	10^6	10^6	10^6	0	
III	Control		0	0	—	—	Normal subject having no natural bactericidal activity. Stock Type V pneumococcus used.
	Pn V Antibody	1 unit per ml	10^6	10^4	10^4	0	
	Pn V Antibody PABA	1 unit per ml 1	10^6	10^5	10^5	0	
	ST	10	10^5	10^5	10^5	0	
	PABA ST	1 10	0	0	—	—	
	Pn V Antibody ST PABA	1 unit per ml 10 1	10^6	10^4	10^4	0	

Growth Inhibition. Numbers represent the largest inoculum which showed no color change in the blood.

Pneumococcidal Action. Listed only when inhibition was noted.

Growth = number of colonies grown in agar pour plates.

Inoculum refers to original number added to the blood. Larger inocula yielded too many colonies to count; smaller ones yielded no growth.

Abbreviations. PABA = para-aminobenzoic acid.

ST = sulfathiazole.

Pn V antibody = concentrated Type V antipneumococcus rabbit serum (Lederle).

h. — hours.

killing power against pneumococci acquired that property on the addition of either sulfathiazole or homologous antipneumococcus rabbit serum. The action of sulfathiazole was completely inhibited by *p*-aminobenzoic acid, whereas the action of the antibody was unaffected by it.

General applicability of this principle seems indicated by the fact that *p*-aminobenzoic acid inhibits the action of other sulfonamide drugs on a wide variety of bacteria.

Conclusions. *P*-aminobenzoic acid inhibits the antibacterial action of sulfathiazole but has no effect on pneumococcidal antibodies in fresh defibrinated blood.

11912

Effect of P-Aminobenzoic Acid on Therapeutic and Toxic Action of Sulfapyridine.*

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In an attempt to determine the chemical identity of the factor responsible for the inhibition of sulfanilamide by certain substances, (*e.g.*, yeast extract, bacterial extracts), Woods[1] reported experiments which demonstrated that *p*-aminobenzoic acid in very high dilution was capable of inhibiting the effect of sulfanilamide on the growth of the *Streptococcus hemolyticus in vitro*. The antibacterial effect of sulfapyridine was similarly impaired, but 5 times as much *p*-aminobenzoic acid was required for inhibiting sulfapyridine as was found necessary to nullify sulfanilamide. The difference was explained by the observation that sulfapyridine was 5 times as potent as sulfanilamide against the strain of *Streptococcus hemolyticus* employed in the experiments. In an accompanying paper from the same laboratories, Selbie[2] demonstrated that *p*-aminobenzoic acid inhibited the therapeutic action of sulfanilamide in mice infected with hemolytic streptococci. Selbie recorded that the survival rate of mice receiving 25 mg doses of sulfanilamide was greatly reduced by administering to the mice as little as 2.5 mg of *p*-aminobenzoic acid. The two drugs were mixed *in vitro* and introduced simultaneously by esophageal cannula.

The present report concerns the inhibiting effect of *p*-aminobenzoic acid on the therapeutic action of sulfapyridine in pneumococcal infection in mice, and also deals with the effect of one drug upon the toxic action of the other.

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* This investigation was supported by funds generously contributed by Mr. Bernard Baruch.

[1] Woods, D. D., *British J. Exp. Path.*, 1940, **21**, 74.

[2] Selbie, E. R., *Ibid.*, 1940, **21**, 90.

Swiss mice weighing 16-20 g were used throughout. Sulfapyridine, suspended in tragacanth mucilage, was introduced by stomach tube in a uniform constant dose of 24 mg to all mice. It was given twice on the day of inoculation and once daily thereafter. *P*-aminobenzoic acid was given subcutaneously in 3 daily doses of equal size, but as may be noted in Table I, for purposes of titration the amount of *p*-aminobenzoic acid differed in individual experiments. Aqueous solution of *p*-aminobenzoic acid was effected by the addition of an equi-molecular quantity of sodium hydroxide. An 18-hour broth culture of a strain of pneumococcus Type I, which regularly induced fatal infection in mice in doses of 10^{-7}, was employed. The dosage of culture, injected intraperitoneally, was kept constant at a dilution of 10^{-4}. The culture was injected immediately after the first dose of sulfapyridine.

In the technical procedures, therefore, it may be noted that the sulfapyridine was given by mouth, *p*-aminobenzoic acid subcutaneously, and pneumococci intraperitoneally. The procedure obviated the possibility of chemical action between the drugs *in vitro*. Treatment was continued for 6 days.

Table I records the results of a series of these experiments. With doses of *p*-aminobenzoic acid, slightly greater or less than the size of the dose of sulfapyridine, the animals died within the same time as the virulence controls. As the dose of *p*-aminobenzoic acid was decreased, the time of death became definitely delayed and more scat-

TABLE I.
Inhibiting Effect of P-aminobenzoic Acid on the Curative Action of Sulfapyridine for Type I Pneumococcus Infection in Mice.

Sulfapyridine (daily dose), mg	P-aminobenzoic acid (total daily dose), mg	No. mice	Deaths Day after infection with pneumococci											Survivals	% survivals
			1	2	3	4	5	6	7	8	9	10	11		
—	—	10	8	2										0	0
24	30	5	1	4										0	0
24	22.5	14	5	8	1									0	0
24	15.0	4	3	1										0	0
24	7.5	14	—	10	2	2								0	0
24	6.0	4	—	—	2	1	—	1						0	0
24	3.0	14	—	2	2	4	1	2	1	—	—	—	1	1	7
24	1.5	14	—	—	4	2	2	2	2	—	—	—	—	2	14
24	0.75	10	—	—	—	1	1	—	—	1	—	—	1	6	60
24	—	24	—	—	—	*1	—	—	†2	—	—	—	—	21	91

*Died during administration of sulfapyridine; postmortem cultures plus for pneumo. I.

†One of these had sterile postmortem cultures.

tered. When the amount of *p*-aminobenzoic acid was reduced to 7.5 mg per day (2.5 mg t.i.d.), there were no deaths on the first day after inoculation, but 10 of the 14 mice succumbed on the second day. At a dosage of 3.0 mg per day the times of death were definitely prolonged and were scattered over a period of several days; one of the mice survived. With further decrease in the amount of *p*-aminobenzoic acid administered, there was a sharp increase in the number of mice surviving the infection. However, even with the smallest dose used (0.75 mg per day) there was some depression of sulfapyridine effect as evidenced by the delayed death of 4 of the 10 mice.

In toxicity experiments, in which no organisms were injected, *p*-aminobenzoic acid caused no untoward symptoms in the doses and by route of administration used in the present study. Furthermore, the combined therapeutic dosage of sulfapyridine plus *p*-aminobenzoic acid, without pneumococcal infection, likewise had no apparent effect on normal mice. Selbie[2] indicated that the toxicity of the combined drugs was somewhat less than that of sulfanilamide alone. In order to determine whether or not the nullifying effect of *p*-aminobenzoic acid on the therapeutic efficacy of sulfapyridine might be accompanied by an alteration in the toxic action of sulfapyridine, the drugs were introduced into uninfected mice according to the titrations indicated in Table II.

The subcutaneous injection of 25 mg of the sodium salt of sulfapyridine (0.5 cc of 5% sol.) in 16-20 g mice caused convulsions proceeding to death in less than 2 hours. *P*-aminobenzoic acid was given by oral dosage of 50 mg a day (in 2 equal doses) for 2 days prior to the injection of the toxic dose of sulfapyridine. There is evidence that *p*-aminobenzoic acid is readily adsorbed from the gastro-intestinal tract in the results of Selbie[2] who demonstrated the effectiveness of small oral doses in nullifying sulfanilamide effect

TABLE II.
Effect of P-aminobenzoic Acid on Acute Toxicity of Sulfapyridine.

P-aminobenzoic acid	Sodium sulfapyridine monohydrate. mg subcut.	No. mice	No. died	No. survived
—	25	9	9	0
50 mg/day *per os* for 2 days	25	6	5	1
25 mg subcut. 30 min. preceding sulfapyridine	25	4	4	0
—	20	3	1	2
50 mg/day *per os* for 2 days	20	3	1	2
—	15	2	0	2
50 mg/day *per os* for 2 days	15	2	0	2

in mice and the studies of Harrow, *et al.*,[3] on the mechanism of detoxification of orally administered *p*-aminobenzoic acid by the formation of the acetylated derivative. However, as a further control in the present experiments, some of the animals were given a single subcutaneous dose of 25 mg of *p*-aminobenzoic acid 30 minutes prior to the injection of sulfapyridine.

The toxic effects of the 25 mg dose of sodium sulfapyridine on normal animals and animals receiving *p*-aminobenzoic acid were compared, and the results of this type of experiment are illustrated in Table II. It will be seen that *p*-aminobenzoic acid exerts no appreciable effect on the acute toxicity of sulfapyridine when administered in this manner. The time of onset and the duration of convulsions in the group receiving *p*-aminobenzoic acid did not differ significantly from that in the control group. The single animal that survived the 25 mg dose of sulfapyridine did so only after several hours of severe convulsions.

Summary. 1. *P*-aminobenzoic acid (given subcutaneously) was found capable of nullifying the curative effect of sulfapyridine (given *per os*) for type I pneumococcus infection in mice. 2. *P*-aminobenzoic acid had no observable effect upon the immediate fatal toxicity of sulfapyridine for mice.

11913

Effect of Intramuscular Injection of Sodium Citrate on the Prothrombin Time of the Blood.*†

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Neuhof and Hirshfeld[1] observed that intramuscular injections of sodium citrate caused an increase in the coagulability of the blood. A fall in the coagulation time occurred within 40 minutes of an intragluteal injection of 30 cc of 30% citrate and was maintained

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[3] Harrow, Benj., Power, F. W., and Sherwin, C. P., Proc. Soc. Exp. Biol. and Med., 1927, **24**, 422.

* This work was supported by the New York Foundation Fund in Experimental Surgery.

† The authors gratefully acknowledge the kind advice of Professor Arthur Wright.

[1] Neuhof, H., and Hirshfeld, L., *Ann. Surg.*, 1922, **76**, 1.

at a lowered level for 1 hour. It returned to its initial value in 24 hours. Hemoglobin estimations, red blood cell counts, carbon dioxide-combining power, oxygen and calcium analyses showed no change after the injection of sodium citrate. The authors accounted for the hypercoagulability on the basis that citrate destroyed the blood platelets from which was liberated a blood-coagulating substance.

De Souza and Hocking[2] investigated the work of Neuhof and Hirshfeld. Their studies, however, followed along the lines of Mills,[3] who had previously demonstrated a direct relationship between alkali reserve and blood coagulation. Thus it was shown that sodium citrate given intramuscularly or intravenously lowered the coagulation time of blood, and that the change was accompanied by an increase in the alkali reserve. Intraperitoneal injections of the salt gave the same effect more slowly, probably due to slower absorption. This excluded the possibility of local muscular damage with release of thromboplastin into the blood being the causative factor in the increased coagulability.

These authors also showed that the alkali reserve was increased by the oxidation of sodium citrate to sodium bicarbonate. Equivalent intramuscular injection of sodium bicarbonate raised the alkali reserve, but the effect on coagulation was more transient. Conversely, ammonium chloride lowered the alkali reserve and raised the coagulation time of the blood. In a series of exclusive experiments it was also shown that hypertonicity of the solution, or the volume injected did not in themselves effect the coagulability of the blood.

Purpose. Due to recent improvements in the method for the estimation of prothrombin it was decided to extend these blood coagulation studies to determine the effect of intramuscular injections of sodium citrate, sodium chloride and potassium chloride on the circulating prothrombin.

Methods. Fourteen normal dogs received, in various concentrations and in different volumes, intramuscular injections of one of the substances mentioned above. Prior to the injection a blood sample was taken on which the following determinations were made: (1) prothrombin time, (2) tube coagulation time, (3) calcium coagulation time, (4) capillary coagulation time, and (5) hematocrit. Following the injection, blood samples were taken every 30 minutes for a period of 2 hours and the estimations repeated. Blood was drawn by needle puncture through the skin from the unanesthetized animal. In addition, 2 control animals which received no injections were subjected to the same tests at the same time intervals.

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[2] de Souza, D., and Hocking, F. D. M., *J. Physiol.*, 1934, **83**, 49.

[3] Mills, C. A., *J. Biol. Chem.*, 1921, **46**, 197.

The prothrombin time determinations were done by the method of Quick. Only freshly prepared thromboplastin was used. The tube coagulation test was performed in a small tube (method of Lee and White). The calcium coagulation time was carried out in the same manner as the Lee and White method except that 3 drops of 1% calcium chloride were previously placed in the tube. The capillary coagulation tests were done in capillaries of uniform bore. Hematocrit determinations were done in the graduated tubes provided for that purpose. During the test period the animals received no water, food or medication.

Results. Six animals whose weight varied from 9 to 17 kg received intramuscular injections of sodium citrate. The amount injected varied from 1.50 g to 8.50 g and averaged from 0.125-0.50 g per kilo. The volume of solution containing this salt was about 50 cc. All solutions were therefore hypertonic.

The initial average prothrombin time was 11.8 seconds. Thirty minutes after the injection the average fall in prothrombin time was 17%. The prothrombin time continued to fall so that at the expiration of 90 minutes the average prothrombin time was reduced 24%. In dog 3, at the end of one hour, the prothrombin time was reduced 50%. The coagulation time as determined by the method of Lee and White was lowered within 60 minutes to 48% of its original value. The calcium coagulation time was carried out in each sample to make certain that the injection of sodium citrate had not materially lowered the calcium content of the blood. Since the results of this test did not vary from the Lee and White method, they

TABLE I.
Effect on Blood of Intramuscular Injection of Various Salts.

Dog	Wt in kg	Injection per kg	Prothrombin time (sec.)					Coagulation time (min.) Lee and White method				
			0	½	1	1½	2 hr	0	½	1	1½	2 hr
1	12.0	Na citrate 0.125g	12.2	9.2	11.2	9.4	9.0	3:25	1:50	1:25	2:05	1:50
2	9.0	" " "	13.0	12.0	12.0	10.0	10.0	4:00	2:25	2:10	2:10	3:00
3	9.0	" " 0.33	12.5	8.0	6.5	9.7	11.0	2:30	1:10	0:30	0:40	1:15
4	17.0	" " 0.50	11.0	11.0	9.0	9.0	10.0	3:20	1:40	1:20	1:40	1:40
5	12.5	" " 0.50	12.0	11.0	9.0	9.0	11.0	3:00	1:30	0:30	1:50	1:50
6	17.0	" " 0.50	10.0	6.6	7.3	7.0	7.0	3:00	1:10	1:40	1:20	1:20
		Avg effect	11.8	9.6	9.1	9.0	9.6	3:12	1:37	1:32	2:02	2:09
7	9.0	Normal dog	9.0	9.0	9.2	9.4	9.5	1:30	1:00	1:40	2:30	2:00
8	14.0	" "	15.5	16.0	15.5	15.5	15.7	2:00	2:40	2:40	2:00	2:00
9	10.5	NaCl 0.64	8.0	8.0	8.0	10.0	8.0	2:30	2:10	2:10	1:30	1:40
10	12.5	" 0.50	11.1	11.9	13.0	12.0	14.2	1:15	1:15	1:15	1:05	0:40
11	11.0	KCl 0.50	8.5	9.2	9.2	9.8	9.2	1:10	2:20	1:50	2:15	1:30

are not shown in Table I. Similarly, the data on the capillary coagulation time are not included in the table, since the results paralleled those of the tube coagulation time. The hematocrit estimations showed no characteristic variations and the results, accordingly, are not given in the table.

In 2 normal dogs used as controls, 5 prothrombin estimations over a 2½-hour period showed no greater variation than 0.5 seconds in the prothrombin time. In addition, 3 other control animals were used; 2 animals were injected with sodium chloride and 1 animal with potassium chloride. None of these animals developed the characteristic fall in the prothrombin and coagulation times, that were invariably obtained when sodium citrate was injected. The hematocrit determinations in the normal controls, as well as in the animals injected with sodium or potassium chloride, showed no characteristic variations.

Summary. Intramuscular injections of sodium citrate caused a lowering of the clotting time of blood as determined by the capillary and Lee and White methods. The increased coagulability was accompanied by a fall in the prothrombin time in the blood, as determined by Quick's method. Control animals, and those injected with sodium or potassium chloride, did not show a lowering of the prothrombin time.

11914

An Error in Measuring Changes in Plasma Volume After Exercise.

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The plasma volume under basal conditions may be satisfactorily determined by the dye method. The original dye method described by Keith, Rowntree, and Geraghty,[1] has been rendered more accurate by the use of better methods of colorimetry and by the calculation of the volume from the disappearance slope of the dye. It is impossible to measure rapid changes in plasma volume which last only a short time by repeated injections of the dye. This is true because changes in plasma volume occurring during the determination dilute or concentrate the residual dye present in the serum from the previous injection. To overcome this difficulty, a method has been developed for measuring rapid fluctuations in plasma volume using a single injection of dye.[2, 3] The disappearance slope of the dye under basal conditions is first determined and changes in optical density above or below this slope, which are induced by experimental procedures, are taken as indicating a decrease or increase in plasma volume. The change in plasma volume is calculated from the ratio of the optical density of the dyed serum to the optical density of the corresponding point on the disappearance slope.

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[1] Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, **16**, 547.

This method has been used to measure the changes occurring in plasma volume after exercise.[4, 5] In studying the effect of exhausting exercise on the plasma volume of human subjects, we noted that the decrease in plasma volume, as measured by this method, did not agree with that obtained by calculating the change in plasma volume from the increase in serum protein concentration. In attempting to find a cause for this discrepancy it was thought possible that the optical properties of the dye-free serum might change during exercise, thus leading to an error. In order to determine whether this occurred, subjects were exercised on a bicycle ergometer to the point of exhaustion. The usual period of exercise was 5 minutes. Samples of blood were taken without stasis before and after exercise. The serum obtained before exercise was compared with the serum obtained after exercise in the Evelyn photoelectric microcolorimeter, using a filter with maximum light transmission at 620 millimicrons.[6, 7] Both samples of serum were also compared with distilled water. It was found that the serum obtained after exercise had a lower galvanometer reading than the serum obtained before exercise. The increase in L value (2—log. galvanometer reading) of the serum after exercise is shown in Table I. A similar increase in Δ D value of serum after exercise was found with the Koenig-Martens spectrophotometer using a light source with a wave length of 620 millimicrons.

The exercise experiments were repeated in 5 cases after the injec-

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[2] Gibson, J. G., 2nd, and Evans, W. A., Jr., *J. Clin. Invest.*, 1937, **16**, 301.

[3] Hamlin, E., and Gregersen, M. I., *Am. J. Physiol.*, 1939, **125**, 713.

[4] Gregersen, M. I., Dill, D. B., and Meade, S., *McLeod's Physiology in Modern Medicine*, p. 929, C. V. Mosby Co., St. Louis, 1938.

[5] Kaltreider, N. L., and Meneely, G. R., *J. Clin. Invest.*, 1940, **19**, 627.

[6] Evelyn, K. A., and Cipriani, A. J., *J. Biol. Chem.*, 1937, **117**, 365.

[7] Gibson, J. G., 2nd, and Evelyn, K. A., *J. Clin. Invest.*, 1938, **17**, 153.

TABLE I.
Effect of Exercise on L Value* of Serum as Measured by the Photoelectric Microcolorimeter.

Subject	Increase in serum protein after exercise (g per 100 cc)	Increase in L value* of serum after exercise
J.G.	1.3	.017
R.E.	1.3	.034
H.W.	.5	.021
M.M.	.7	.005
L.H.	1.2	.013
R.E.	.8	.019
Avg	0.96	.018

*L value = 2 — log. of galvanometer reading.

tion of dye, in order to decide whether the change in the optical properties of the dye-free serum caused a significant error. Using 10 mg of Evans blue, the average increase in L value of the serum after exercise was .045. The average change in L value of the serum of the subjects who had not received dye was .018. Thus, 40% of the change in L value usually attributed to an increase in dye concentration was actually due to a change in the L value of the serum. The decrease in plasma volume which occurs after exercise is exaggerated by the dye method. This error has not been taken into account by recent workers using the dye method for determining changes in plasma volume after exercise.[4, 5] If this method is to be used for determination of changes in plasma volume produced by other procedures than exercise, it must first be demonstrated that the optical properties of the dye-free serum are not altered by the procedures.

The error caused by the change in optical properties of the serum varies inversely with the amount of dye used. If 20 mg of dye had been injected instead of 10 mg, the error from the change in optical properties of the serum would have been reduced by half. It is not practical to use more than 20 mg of Evans blue in human subjects, because larger quantities of the dye may cause discoloration of the skin. In animal experiments, however, this error can be minimized by using larger amounts of dye.

Summary. When the dye method is used for measuring changes in plasma volume after exercise, an error occurs due to changes in the optical properties of the serum.

11915

Effect of Dosage on the Morphogenetic Actions of Testosterone.

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The literature concerning many of the morphogenetic actions of steroid hormones in general and testosterone in particular is rich in contradictions. In the course of recent investigations performed in this laboratory it became increasingly more evident that much of the confusion concerning these actions is due to differences in the dosage in which the steroids were administered. While some morphogenetic actions are proportional to the dose, others are not; in fact the same compound may either stimulate or inhibit the development of an organ depending on the dose used. Space does not permit a complete discussion of the voluminous relevant literature here, so that the reader is referred for reviews to more detailed papers on cognate subjects.[1, 2, 3]

In an effort to obtain a clearer picture of the effect of dosage on the morphogenetic action of testosterone we performed the following experiment on 36 five-week-old male albino rats which were divided into 6 groups of 6. The rats in 5 of these groups were injected once daily with a very fine suspension of testosterone in 1 ml of physiological NaCl solution during a period of 20 days. In order to obtain good absorption the material was injected subcutaneously and intraperitoneally, the site of administration being varied on alternate days. An aqueous suspension was given, instead of the usual solution in oil, in order to avoid the administration of the enormous quantities of oil which would have been necessary to dissolve the amount of testosterone given in some of the groups. Biopsy at the end of the experiment indicated that, except for traces in the groups receiving the highest doses, the hormone was well absorbed from the site of injection. On the 21st day all animals were killed, their organs dissected and weighed after complete fixation in Heidenhein's "Susa" solution. Table I summarizes our results. Each figure represents the average of 6 animals. The significance of the apparent differences between the treated and control groups was evaluated by "Student's method for small samples"[4] and is expressed

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[1] Selye, H., and Friedman, S., *Endocrinol.*, 1941, **28**, 129.

[2] Selye, H., and Friedman, S., *Endocrinol.*, 1940, **27**, 857.

[3] Selye, H., *J. Am. Med. Assn.*, 1940, **115**, 2246.

[4] Fisher, R. A., *Statistical Methods for Research Workers*, 6th ed., Edinburgh, 1936, p. 128.

TABLE I.
Effect of Dosage on Morphogenetic Actions of Testosterone.

Organ	Saline	Testosterone dose				
		0.1 mg	0.5 mg	1.0 mg	10 mg	25 mg
Initial body wt (g)	80	70	81	75	85	73
Weight gain (g)	+69	+75	+64	+61	+*42*	+*30*
Pituitary wt (mg)	5.5	5.3	5.1	*4.4*	*4.2*	*4.0*
Thyroid wt (mg)	14.0	12.0	*12.0*	*12.0*	*11.0*	*11.0*
Adrenal wt (mg)	31.0	31.0	*26.0*	*25.0*	*37.0*	*43.0*
Thymus wt (mg)	280	353	288	226	*49.0*	*15.0*
Testis wt (g)	2.13	*1.46*	*1.29*	*1.22*	1.74	1.92
Seminal vesicle wt (mg)	125	86	*231*	*467*	*1116*	*1004*
Prostate wt (mg)	301	247	357	*464*	*837*	*655*
Epididymis wt (mg)	279	205	289	292	*392*	*462*
Kidney wt (g)	1.45	1.48	1.44	1.37	1.56	1.40

in terms of probability estimated by graphic interpolation in Fisher's table of *t*. In accordance with the usual convention, differences between series cannot be accepted as significant when *P* is greater than 0.05. All values in which the apparent deviation from the normal control level proved to be statistically significant in this sense, are indicated in italics in the table.

As seen from the table testosterone caused a decrease in the size of the *pituitary*. This became increasingly more pronounced as the dose of the hormone was raised. The hypophyseal atrophy was statistically significant at the 1 mg per day dose level, although this amount of testosterone did not cause any significant inhibition of body growth. We may conclude from this that the pituitary-depressing action of the hormone[5, 6] is specific and not merely a repercussion of the general inhibition of body growth. Histologically the most prominent feature of the atrophic glands is their poverty in eosinophil cells, combined with a relative increase in the number of small chromophobes. The *thyroid* showed a significant decrease in weight with as little as 0.5 mg of testosterone daily, that is long before a general body growth depressing dose had been reached, so that here again the decrease in thyroid size cannot be regarded merely as the result of general growth inhibition. Histological examination shows that this decrease in size is accompanied by definite atrophy of the follicular epithelium, which, like the decrease in the weight of the gland, is roughly proportional to the dose in which testosterone was given. The fact that suitable doses of testosterone cause thyroid atrophy in the rat has already been emphasized in a previous publication.[5] The hormone has also been shown to cause thyroid atrophy in the mouse.[6]* The *adrenals* show a statistically significant decrease in weight at the 0.5 and 1.0 mg levels, while larger doses result in an equally significant increase in weight. It is obvious that here the result is not simply proportional to the dose in which the hormone is given but that there is a definite optimum range for the production of atrophy. This may explain the contradictory findings published on this subject by other investigators and recently reviewed by us.[3] It also confirms our earlier reports on the production of adrenal atrophy with androgens in the rat[7, 5] and mouse.[6] Histological examination showed the atrophy to be due to a more or less uniform decrease in the size of the cells in the various layers of the adrenal cortex. In this respect the response differed from that obtained in extremely young rats in which testosterone causes predominantly glomerulosa atrophy.[8] It also differs from the involution caused by hypophysectomy which affects the reticularis more markedly than the other zones. In almost all the animals in which a decrease in adrenal weight was obtained the chromaffine cells of the medulla did not participate to any extent in the involution, but the ganglion cells in the medulla often show a peculiar transformation characterized by the appearance of large vacuoles inside the cytoplasm. The nerve cells affected in this manner were strikingly similar to the "signet ring" or "castration cells" which appear in the anterior lobe of the rat after spaying. The increase in adrenal weight caused by high doses of testosterone is mainly due to cortical enlargement. It should be emphasized, however, that this adrenal hypertrophy occurs only with doses which are definitely damaging as shown by the decrease in body weight. It is quite possible, therefore, that the hypertrophy of the cortical cells should merely be regarded as part of the reaction to non-specific damage, a reaction which is always accompanied by adrenal cortical enlargement.[9] The *thymus* shows progressively increasing atrophy which runs closely parallel with the dose of testosterone given. Histologically this decrease in weight proves to be due mainly to disappearance of thymocytes. The *testis* shows the most pronounced atrophy with a dose of 1 mg per day, but as little as 0.1 mg suffices to cause a statistically significant testis involution. However, doses of 10 mg a day no longer cause a statistically significant decrease in testis weight while the animals treated with 25 mg per day had testes which were almost back to normal, a fact which is all the more remarkable since the body weight in the 25 mg group was only 103 g on the average, as compared with 149 g in the controls. This observation gives strong support to the view[1] that large doses of androgens may exert a direct stimulating action on the testis. Histologically the interstitial cells showed progressive atrophy with increasing dosage irrespective of testis weight, but the germinal epithelium revealed marked atrophy only in the 0.1-1.0 mg range. The apparently slightly subnormal size of the *accessory sex organs* (seminal vesicles, prostate and epididymis) in the 0.1 mg group did not prove to be statistically significant and may perhaps be due to the somewhat smaller average size of the animals in this group. Otherwise the increase in the size of these organs became more and more pronounced as the dose was increased. Contrary to expectations there was no statistically significant increase in *kidney* weight in any of the groups of this series, although our previous observations[10, 6, 5, 11] which have amply been confirmed in other laboratories[12-15] definitely indicate that androgens have a renotropic action. The reason for this failure to obtain a more pronounced enlargement in kidney size is probably that, as we have repeatedly emphasized, the rat is not very sensitive to the renotropic action of steroids. Furthermore, we used intact males in this experiment and in these the kidney size is relatively large as compared with that of females or spayed animals of either sex, so that a further increase in size is difficult to obtain. It will be noticed, however, that in the 10 mg group the average kidney size was above normal although the average body weight was below that of the controls and histological study of this material confirmed the existence of a definite, though mild degree, of tubular hypertrophy.

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[5] Selye, H., *Canad. Med. Assn. J.*, 1940, **42**, 113.

[6] Selye, H., *J. Endocrinol.*, 1939, **1**, 208.

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* Attention is called to an error in the printing of the latter communication in which the explanatory remarks illustrating the microphotographs of the testosterone-treated and control thyroids were exchanged. This gives the impression that testosterone stimulates thyroid growth although the tables in that paper, giving each individual thyroid weight, definitely proved the contrary to be true.

[7] McEuen, C. S., Selye, H., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 390.

[8] Selye, H., *Anat. Rec.*, 1940, **76**, 145.

[9] Selye, H., *Endocrinol.*, 1937, **21**, 169.

Summary. Young male albino rats were divided into groups treated with daily doses of testosterone ranging from 0.1 to 25 mg per day. These experiments showed that the body weight as well

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[10] Selye, H., *J. Urol.*, 1939, **42**, 637.

[11] Selye, H., *Anat. Rec.*, 1941, in press.

[12] Korenchevsky, V., and Ross, M. A., *Brit. Med. J.*, 1940, **1**, 645.

[13] Ludden, J. B., and Krueger, E., *J. Clin. Invest.*, 1940, **19**, 780 P.

[14] Paschkis, K. E., Shay, H., Gershon-Cohen, J., and Fels, S. S., *Am. J. Physiol.*, 1940, **129**, 191.

[15] Pfeiffer, C. A., Emmel, V. M., and Gardner, W. U., *Yale J. Biol. and Med.*, 1940, **12**, 493.

as the weight of the pituitary, thyroid and thymus undergo a gradual decrease as the hormone dosage is increased. The seminal vesicle, prostate and epididymis increase in size with increasing dosage. On the other hand, the inhibitory action of testosterone on the adrenal and testis has a definite optimum at the 0.5 to 1.0 mg daily dose level, inasmuch as smaller doses cause less pronounced atrophy and larger amounts may actually stimulate the growth of these glands. The histological changes which accompany these variations in gross weight have been described. We conclude that many of the apparent contradictions concerning the morphogenetic actions of androgens—and perhaps also of other steroid hormones—are due to the fact that depending on the dose given, these compounds may exert diametrically opposite actions on certain organs.

The expenses of this investigation were defrayed through a grant in aid received from the Cooper Fund of McGill University. The author is especially indebted to Drs. G. Stragnell and E. Schwenk of the Schering Corporation of Bloomfield, New Jersey, for supplying the large quantities of testosterone required for this work.

11916

Riboflavin Content of Blood and Muscle in Normal and in Malnourished Humans.*

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A number of workers have reported significant decreases in the riboflavin content of tissues from experimental animals maintained on a riboflavin deficient diet.[1-6] The resultant conclusions, however, cannot safely be applied to man.

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* University of Cincinnati Studies in Nutrition at the Hillman Hospital, Birmingham, Alabama. These studies were aided by grants from the John and Mary R. Markle Foundation and the Wisconsin Alumni Research Foundation.

[1] Axelrod, A. E., Sober, H. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **134**, 749.

[2] Axelrod, A. E., Lipton, M. A., and Elvehjem, C. A., *Am. J. Physiol.*, in press.

[3] Kuhn, R., Kaltschmitt, H., and Wagner-Jauregg, T., *Z. physiol. Chem.*, 1935, **232**, 36.

[4] Groen, J., and Schuyl, J. W., *Arch. néerl. physiol.*, 1938, **23**, 271.

[5] Carlsson, E. V., and Sherman, H. C., *J. Nutrition*, 1938, **15**, 57.

[6] Fraser, H. F., Topping, N. H., and Isbell, H., *Pub. Health Rep., U. S. P. H. S.*, 1940, **55**, 280.

In this study we have made a comparison of the blood and muscle riboflavin values in a group of normal subjects and in a group of malnourished persons selected from the Nutrition Clinic, Hillman Hospital, Birmingham, Alabama.

Blood Studies. One blood sample was taken before therapy from each of 35 patients with varying degrees of riboflavin deficiency and from 30 members of the laboratory staff. A diagnosis of riboflavin deficiency in these patients was based on the presence of cheilosis and conjunctivitis which, in each instance, were relieved by the oral administration of riboflavin. Five ml of blood were obtained by venepuncture and potassium oxalate was used as the anticoagulant. Precautions were taken to avoid exposure to light and the blood was stored in the refrigerator occasionally for a 2-week period as no diminution in the riboflavin content due to storage under these conditions was ever observed.

The riboflavin content of blood was determined according to the microbiological method of Snell and Strong.[7] In this method, which is based upon the fact that riboflavin is essential for the growth of a specific strain of *Lactobacillus casei*, the growth of the organism, as measured by the acid production, is proportional to the amount of riboflavin added to a riboflavin-deficient medium. The reliability of this method for the determination of both the free and the combined forms of riboflavin in a number of biological fluids has been established definitely by a variety of tests.[7, 8, 9] The blood was hemolyzed immediately before the analysis and aliquots of hemolyzed blood equivalent to 0.1 and 0.2 ml of whole blood were added to assay tubes in duplicate. The addition of larger amounts of hemolyzed blood yielded lower values of riboflavin when calculated per ml of the original blood, a phenomenon which suggested the presence of inhibiting substances in appreciable quantities when amounts of hemolyzed blood equivalent to more than 0.2 ml of whole blood were added. The results obtained when 0.2 ml of whole blood or less were used per assay tube were considered accurate since recovery experiments at these concentrations proved to be satisfactory.

The blood riboflavin values of the 20 normal subjects ranged between 0.35 and 0.45 μg per ml with an average value of 0.42 μg per ml. In the blood of the patients with clinical evidence of riboflavin deficiency, the range of values was the same as in the control group with an average value of 0.40 μg per ml. There was no indication that the concentration of riboflavin in the blood decreased as the deficiency lesions became more severe.

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[7] Snell, E. E., and Strong, F. M., *Ind. and Eng. Chem.* (Analyt. Ed.), 1939, **11**, 346.

[8] Feeney, R. E., and Strong, F. M., *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.*, 1940, **133**, 31.

[9] Strong, F. M., Feeney, R. E., Moore, B., and Parsons, H. T., *J. Biol. Chem.*, 1941, **137**, 363.

Muscle Studies. Muscle specimens were obtained by biopsy from 30 patients who were chosen because they represented a group with varying degrees of pellagra and interrelated vitamin deficiencies. Our prime interest in these cases was the study of the coenzyme I content of muscle as related to the degree of nicotinic acid deficiency. This group, although presumably riboflavin deficient to some degree, showed no striking clinical manifestations of this specific deficiency. Muscle specimens obtained by biopsy from 9 patients of normal nutrition who were undergoing a herniorrhaphy at the Hillman Hospital served as a control group.

The section of the quadriceps femoris muscle, removed at biopsy, was immediately frozen on carbon dioxide snow. A portion of this frozen tissue was kept for moisture determinations and another sample was ground to a fine powder in a mortar which was kept at the temperature of the carbon dioxide. No significant variation in moisture content was observed. The finely pulverized tissue (0.5-1.0 g) was weighed on a cold watch glass and washed with approximately 5 cc of hot water into 25 cc of boiling water. The extract was boiled for 3 minutes and cooled immediately. These precautions were taken in order to preserve the coenzyme I content of the tissues and were probably not necessary in the riboflavin determination. The coenzyme I content was determined in an aliquot of this extract[10] and the remainder was stored in the refrigerator under toluene and later used for the determination of riboflavin according to the microbiological method of Snell and Strong.

The riboflavin contents of the muscles from the control group ranged between 2.2 and 3.5 μg per gram of fresh weight, with an average value of 2.9 μg per gram. The values for the 30 patients in the malnourished group also lay within the same range with an average value of 2.8 μg of riboflavin per gram of muscle.

Summary and Conclusions. 1. The blood riboflavin values of 20 normal subjects ranged between 0.35 and 0.45 μg per ml, with an average value of 0.42 μg per ml. The same range of riboflavin values was found in a group of 35 patients with varying degrees of riboflavin deficiency. The average value for this group was 0.40 μg of riboflavin per ml.

2. The muscle riboflavin values of 9 control subjects ranged

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[10] Axelrod, A. E., Spies, T. D., and Elvehjem, C. A., *J. Biol. Chem.*, in press.

between 2.2 and 3.5 μg per gram of fresh weight with an average value of 2.9 μg per gram. The muscle riboflavin values for 30 pellagrins lay within the same range with an average value of 2.8 μg per gram of fresh muscle.

3. From these results we have concluded that a determination of blood and muscle riboflavin values is of little significance in the evaluation of a state of riboflavin deficiency in man.

11917

Assay of Secretin.

HARRY GREENGARD AND IRVING F. STEIN, JR. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Recently a secretin preparation designed for human use was developed and tested clinically in normal individuals and in patients with pancreatic and biliary tract disease.[1, 2, 3] Prepared by an unpublished method, this product is now manufactured and sold under the name *Pancreotest* by the Astra Laboratories, Sodertalje, Sweden. Through the courtesy of Messrs. Astra, a sample of Pancreotest was made available to us. This particular material had been produced recently (May 1, 1940) and was standardized by the manufacturer against the crystalline secretin of Hammarsten, *et al.*[4] Having this standardized product we could evaluate its potency in relation to that of our own secretin preparations and investigate an apparent discrepancy in the expression of secretin units. Assays were conducted on 5 dogs by the technic previously described.[5]

Results. Pancreotest was found to be free of vasodilator substances when injected intravenously in amounts up to 10 mg. Weight for weight it was about half as potent as our standard SI preparation in stimulating the pancreas to secrete.[5] The data on the 5 dogs are given in Table I.

Discussion. As is evident from the table, a given amount of Pan-

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[1] Agren and Lagerlof, *Acta med. Skand.*, 1936, **90**, 1.

[2] Agren and Lagerlof, *Acta med. Skand.*, 1937, **92**, 359.

[3] Lagerlof, *Quarterly J. Med.*, 1939, **8**, 115.

[4] Hammarsten, Agren, Hammarsten, and Wilander, *Biochem. Z.*, 1933, **264**, 272.

[5] Greengard and Ivy, *Am. J. Physiol.*, 1938, **124**, 427.

TABLE I.
Secretory Response to Weighed Amounts of SI and of Pancreotest.

Dog No.	SI injected (mg)	Pancreatic juice (drops)	Pancreotest injected (mg)	Pancreatic juice (drops)
1	1.0	17	1.0	10
	0.5	7	0.5	2
	0.5	8	1.0	7
2	0.5	36	0.5	20
	0.1	7	0.1	0
	0.4	15	0.8	17
3	0.5	19	1.0	20
	0.25	7	0.5	7
4	0.5	3	0.5	0
	1.0	8	1.0	2
	2.0	21	2.0	9
5	0.25	7	0.5	8
	0.5	16	1.0	17
	1.0	38	2.0	36

creotest is equivalent to half the same amount of SI in stimulating the pancreas to secrete. The corollaries to this finding are several: (a) The sample of Pancreotest was stated by the manufacturer to contain 2 "clinical units" per milligram which in turn is equivalent to 32 "cat units" as defined by Wilander and Agren.[6] Our assays indicate the material to contain 2 threshold doses or "dog units" per milligram. Hence one "dog threshold dose" equals 16 cat units. (b) In a previous communication[5] we reported that one "dog unit" is equal to 20 Wilander-Agren "cat units". Our Pancreotest assays show this ratio to be smaller, or about 1:16. The discrepancy is probably explained as follows: Our[5] comparisons were made on the basis of cat's pancreatic juice obtained by direct cannulation. The Swedish investigators collected mixed secretions from the lumen of the duodenum. (c) The crystalline secretin of Hammarsten, *et al.*, has a reported potency of 253 cat units per milligram. On the basis of 16 cat units per "dog threshold dose", this is equivalent to 15 or 16 dog threshold doses per milligram, and the "dog threshold dose" of the Hammarsten preparation is therefore about 0.065 mg. This is approximately the same potency as our own crystalline secretin picrolonate, and these observations check well with our[5] previously reported potency of 0.0036 mg (secretin picrolonate) according to our modification of the Wilander-Agren cat assay method. It should be emphasized again that our crystalline secretin picrolonate contains about 80% picrolonic acid, whereas this constituent comprises only a small portion of the Hammarsten preparation. The "dog threshold dose" for our secretin base is 14 gamma.[5] (d) The preparation, Pancreotest, is about half as potent as our standard SI material, which we have considered to be too imperfect for use in humans. The secretin used by us[7] on human subjects is 10 times as potent as our SI and 20 times as potent as Pancreotest.

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[6] Wilander and Agren, *Biochem. Z.*, 1932, **250**, 489.

Conclusions. The commercial secretin preparation, Pancreotest, has been assayed on dogs and found to be half as potent as our standard material. An apparent discrepancy in the expression of units of secretin has been explained.

11918 P

Electrolytic Splitting of Liver Albumin.

J. MURRAY LUCK, C. NIMMO AND C. ALVAREZ-TOSTADO.

From the Biochemical Laboratory, Stanford University.

The albumin used in these studies was prepared from dog liver perfused *in situ* until free from blood. In all cases the animals had been well fed. The organ was excised, frozen in liquid air, powdered, and stored at –13°C. Preparation of the liver albumin followed upon extraction of the powdered liver with 0.5 M $(NH_4)_2SO_4$, and salting out of the globulins in 2.1 M $(NH_4)_2SO_4$. The albumin was salted out by increasing the concentration of ammonium sulphate to 3.5 M and was rendered free from any detectable amount of globulin by reprecipitation with ammonium sulphate. After 3 or 4 reprecipitations, the amount of material salted out in 2.1 M $(NH_4)_2SO_4$ was reduced to the vanishing point. The pH was maintained at 6.5 to 7.0 throughout this treatment. The amount of albumin obtained from 100 g of liver was 0.9 to 1.5 g.

Electrophoretic studies were carried out in the Tiselius apparatus, using for observation of the boundaries the Toepler "Schlieren" method as modified by Longsworth. In a medium consisting of 0.1 M NaCl and 0.02 M KH_2PO_4/K_2HPO_4, following upon dialysis against a fluid of the same composition, electrophoretic analysis (pH 5.8 to 7.3) revealed 2 fractions; both moved towards the positive electrode. One fraction was considerably larger than the other and moved more slowly.* The other fraction, small in quantity, has shown much variability; in some cases it was practically absent. This fraction is isoelectric at pH 4.7 and we suspect it may consist of traces of serum albumin not washed out during perfusion of the liver.

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[7] Voegtlin, Greengard and Ivy, *Am. J. Physiol.*, 1934, **110**, 198.

The major fraction has always displayed much stability and homogeneity in media consisting of 0.1 *M* NaCl and 0.02 *M* in either $PO_4^{\equiv}$, Ac^-, or $PO_4^{\equiv}$ and Ac^-, according to the pH; many runs have been made over a wide range of pH (3.9 to 8.0) without evidence of heterogeneity. After about 7 hours of electrophoresis at 2.0 to 2.5 volts/cm the band tends to become somewhat diffuse at the edges but there is no evidence of splitting.

In potassium phosphate buffers of ionic strength equal to or slightly less than that used in the sodium chloride experiments ($\mu = 0.2$) the stability of the albumin is much reduced. In 8 experiments on albumin prepared from livers from 3 different dogs, after electrophoresis was continued for 3 to 4 hours at pH 5.4 to 6.7 and 2.0 to 2.5 volts/cm, the major band split suddenly and rapidly so that within 30 minutes as many as 4 or 5 rapidly moving components arose from the albumin.

In our opinion this is a true splitting of the protein, although admittedly there is much to be done to further elucidate the phenomenon. The appearance of multiple bands is not due to convection since control experiments with sucrose[1] have shown that convection bands do not appear unless the input of power be 3 to 5 times greater than was employed in these experiments. Individual variations can also be ruled out, since albumin prepared from any given liver was homogeneous in the low phosphate medium and produced multiple bands in the high phosphate buffer. It deserves mention that this splitting is electrolytic in character. Though dependent upon the presence of phosphate, prolonged standing of the protein in the phosphate medium is not in itself sufficient to ensure dissociation of the protein. An electrical field seems to be essential. Under the conditions of our experiments the passage of current for 3 to 4 hours is required.

Finally, there is some indication that the occurrence of this splitting depends on the nutritional state of the animal. Three liver albumin preparations did not show the splitting but the dogs from which these have been prepared had been starved 3 days and given epinephrine (0.2 mg/kilo) one to two hours before removal of the liver. Liver albumin from 2 dogs which we had supposed normal did not give a completely characteristic splitting, yet did not give the single band which the liver albumin from starved epinephrinized dogs showed. We have reason to believe from other evidence that these 2 dogs were not in a normal nutritional state.

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* Isoelectric point, pH 5.6 to 5.8.

[1] Alvarez-Tostado, C., *J. Biol. Chem.*, 1940, **135**, 799.

11919 P

An Electrophoretically Homogeneous Component of Ragweed Producing Hay Fever.*

HAROLD A. ABRAMSON, D. H. MOORE AND H. H. GETTNER.

From the Electrophoresis Laboratory, Department of Physiology, College of Physicians and Surgeons, Columbia University, and the Laboratories, Mount Sinai Hospital, New York City.

It has been shown[1, 2] that in extracts of giant ragweed pollen there is a major component which is unpigmented and which moves slowly in the electrical field at pH 7.4 in phosphate buffer. In addition to this unpigmented, slow component (designated as US) as many as 6 other components, probably pigments, were observed by means of the electrophoretic technic. These electrophoretic studies have now been extended to dwarf ragweed extract. Ultracentrifugal and diffusion data have also been obtained.

Figs. 1a and 1b represent the electrophoretic patterns obtained with crude giant and crude dwarf ragweed extract in M/6 phosphate buffer at pH 7.4. Note the similarity of the two sets of curves which were obtained with the Philpot-Svensson technic. The migration time was 2 hours. The base line on the right side of each of the figures has been obliterated. In both ascending and descending parts of the electrophoresis cell, the major component and pigments correspond fairly well. Not all of the pigments are shown.

The giant component† (USG) and the dwarf component† (USD were isolated in the Tiselius cell and studied in the ultracentrifuge.

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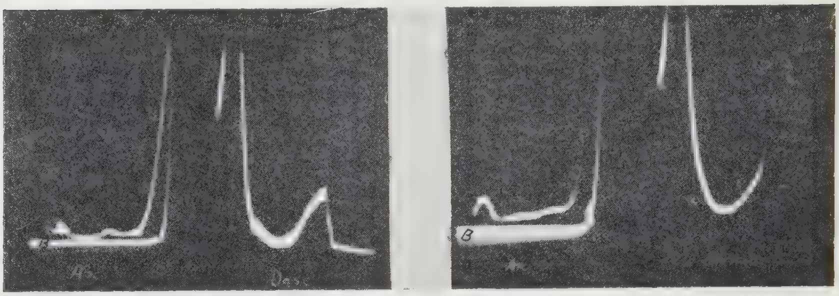
* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Abramson, H. A., Moore, D., Gettner, H., Gagarin, J., and Jennings, L., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 311.

[2] Abramson, H. A., and Moore, D. H., *J. Lab. and Clin. Med.*, 1940, **26**, 174.

† The names Trifidin and Artefolin are suggested for the USG and USD components respectively.



a b

Fig. 1.

Figs. 1a and 1b represent the electrophoretic patterns obtained with crude giant ragweed extract and crude dwarf ragweed extract in M/6 phosphate buffer at pH 7.4. Note the similarity in the two curves. The photographs (using the Philpot-Svensson technic) have been made after two hours of migration. In both ascending and descending parts of each of the figures, the major components and pigments are somewhat the same. The base lines have been removed on one side of each.

These colorless components were also found to be monodisperse in the ultracentrifuge and to have sedimentation constants of $1.5 \pm 0.2 \times 10^{-13}$ at room temperature. In addition, crude giant and dwarf extracts were ultracentrifuged. The sedimentation rate of the boundaries observed in the crude extracts corresponded very closely to the rate of the USG and USD components although some heterogeneity may have been present. However, with a force of about 150,000 times gravity much of the pigment remained in solution. The pigment intensity was apparently the same above and below the descending boundaries.

The USG component was sprayed into the nose in 15 hay fever cases and 7 normals. None of the normals reacted to the USG component. Nine out of the 15 cases of hay fever responded with symptoms varying from mild hay fever to severe hay fever and asthma.

The USD component is highly skin reactive (slight reaction on scratch test with a solution containing 0.0003 mg N/cc) and its reactivity is of the same order of magnitude as that of the USG component reported previously.

Preliminary data obtained for the diffusion constant of the USG component shows that it diffuses rapidly compared with a protein like serum albumin. The data thus far obtained at 2°C indicate that D is $1.7 \pm 0.5 \times 10^{-6}$ cm^2 per sec. The rapidity of diffusion and sedimentation indicates that the major colorless components have low molecular weights when compared with proteins. Indeed, the calculation of the molecular weight using our values for the sedimentation and diffusion constants gives a molecular weight for the USG component of about 5000. This low value of the molecular weight fits in with the fact that biologically active material is readily introduced into the skin by electrophoresis.[3]

The low value of the molecular weight suggests that it may belong to a special group of allergenic substances of low molecular weight found in the pollens. The USG component gives a white precipitate with phosphotungstic acid, a Biuret reaction, and a Millon test. It is not coagulated by boiling. There was no precipitate with trichloracetic acid, nitric acid, or sulphosalicylic acid. (The Molisch test was negative.) The USG component is, therefore, not a protein. It is possible that its properties correspond with a very high molecular weight polypeptide or peptone. The solution tested contained 0.24 mg of nitrogen per cc.

The fast moving pigment and the pigment mixtures have not been investigated in detail but skin reactivity has been found in solutions of pigments presumably free of the USG and USD components. Passive transfer experiments are in progress.

Summary. Ultracentrifugal and diffusion studies of the major colorless components of both giant and dwarf ragweed extracts indicate that these major components are of fairly low molecular weight and do not have the ordinary chemical reactions of proteins. The low molecular weight which fits in with the high biological activity is in accord with the ability of these molecules to enter the mucous membranes, produce hay fever, and be transported into the skin by electrophoresis.

11920 P

Effects of Choline, Gelatin and Creatine on Perosis in Chicks.

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From the Division of Poultry Husbandry, University of California, Davis.

A basal diet consisting principally of glucose, casein and yeast was used[1, 2] for the production of choline deficiency in turkeys. The deficiency was characterized by perosis and slow growth. It was later found that when such a diet was fed to chicks, perosis did not develop, and little or no increased growth was produced by adding choline to the diet. However, when part of the casein and glucose in the basal diet was replaced by gelatin and gum arabic to supply the "rice factor",[3] perosis was produced. If choline was supplied in addition to the "rice factor," perosis was prevented and growth was greatly stimulated. Thus it appeared that some component of the "rice factor" was necessary for the production of perosis in chicks and also for the growth-promoting effect of choline to be exerted.

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[3] Abramson, H. A., *Science*, 1938, **87**, 299; Abramson, H. A., *N. Y. State J. Med.*, 1939, **39**, 1611; Abramson, H. A., and Gorin, M. H., *Cold Spring Harbor Symposia*, 1940, in press.

[1] Jukes, T. H., *J. Biol. Chem.*, 1940, **134**, 789.

[2] Jukes, T. H., *J. Nutrition*, 1940, **20**, 445.

Gelatin and gum arabic, supplying the two components of the "rice factor", were then fed separately and in combination, and the effects described above were found to be due to gelatin and not to gum arabic, although gum arabic was necessary for maximum growth. Furthermore, the growth-promoting effect of gelatin was greatly reduced unless choline was present in the diet. The following basal diet was then used to study the relation between gelatin and choline: glucose ("Cerelose"), 53 parts; washed casein, 18; brewers' dried yeast (Anheuser-Busch, Strain G), 6; gum arabic, 5; salt mixture,[4] 5; crude soybean oil, 5; fish oil concentrate (3000-A, 400-D), 0.3. Eight chicks were used in each group. The feathers of the chicks not receiving gelatin were rough and staring. The findings are illustrated in Table I.

The work of Almquist and Mecchi[5] suggested that creatine could replace gelatin. When creatine was added to the above basal diet, the results obtained were similar to those described with gelatin in

TABLE I.
Effects of Gelatin and Choline on Growth and Perosis in Chicks.

Addition to 92 g of basal diet	No. of chicks showing perosis at 21 days	25 days	Gain in g during first 25 days
8 g casein	1*	0	57
" " + 0.1 g choline chloride	0	0	78
" gelatin	7	7	76
" " + 0.02 g choline chloride	4	3	110
" " + 0.04 " " "	4	3	133
" " + 0.07 " " "	1*	0	134
" " + 0.1 " " "	0	0	144

*Mild.

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[3] Stokstad, E. L. R., and Manning, P. D. V., *Poul. Sci.*, 1939, **18**, 413; Almquist, H. J., Stokstad, E. L. R., Mecchi, E., and Manning, P. D. V., *J. Biol. Chem.*, 1940, **134**, 213.

[4] Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 180.

[5] Almquist, H. J., and Mecchi, E., *J. Biol. Chem.*, 1940, **135**, 355.

Table I. Creatine produced perosis, which was prevented when choline was also added. Almquist and Mecchi[5] showed that a dietary deficiency of creatine or its precursors results in muscular dystrophy in chicks. It may be speculated that the tension exerted on the bones by the muscles plays a part in causing the distortion of the bones which characterizes perosis. If the tension is reduced by muscular dystrophy as in creatine deficiency, the tendency towards perosis may be lessened.

Summary. In contrast to turkeys, chicks on a simplified diet, deficient in choline, did not develop perosis unless gelatin or creatine was added. Choline prevented the perosis at a level of 0.1%, but lower levels were only partially effective. A possible explanation for the perosis-producing effect of gelatin or creatine is advanced.

11921

Vago-Insulin and Sympathetico-Adrenal Systems, Their Mutual Relationship. II. Reaction to Cocaine and Bulbocapnine.*

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For over 60 years (Von Anrep[1]) the action of cocaine on the sympathetic nervous system has been known. Several studies (Bömer,[2] Oelkers and Schutze[3]) have shown that in normal animals subcutaneous injection of cocaine produces hyperglycemia. The reactions of bulbocapnine, on the other hand, suggest marked parasympathetic stimulation (Molitor,[4] Kolb and Langworthy[5]) yet here also hyperglycemia is produced in normal animals (Sarno[6]).

In the first paper of this series (Feldman, Cortell and Gellhorn[7]) we have shown that under the influence of anoxia and metrazol both sympathetic and parasympathetic centers are stimulated. It seemed

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* Aided by a grant from the John and Mary R. Markle Foundation and W.P.A. Project 30278.

[1] Von Anrep, B., *Pflug. Arch. ges. Physiol.*, 1880, **21**, 38.

[2] Bömer, M., *Arch. exp. Path. Pharmak.*, 1930, **149**, 247.

[3] Oelkers, H. A., and Schutze, G., *Klin. Wschr.*, 1938, **17**, 871.

[4] Molitor, H., *J. Pharm.*, 1938, **62**, 16.

[5] Kolb, L. C., and Langworthy, O. R., *J. Pharm.*, 1938, **63**, 108.

[6] Sarno, D., *Bull. Soc. Ital. Biol. Sper.*, 1931, **6**, 714.

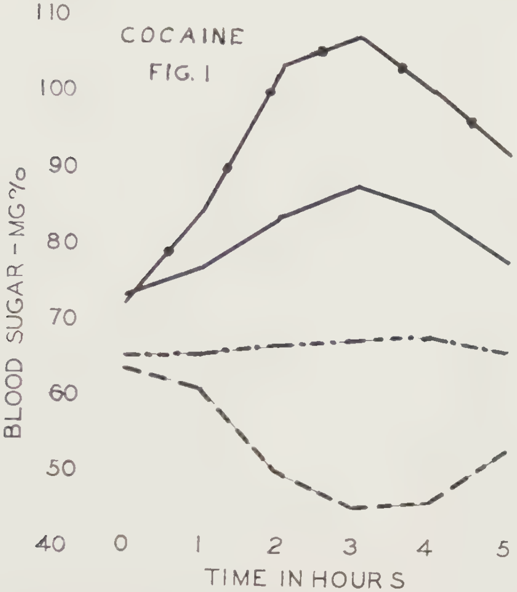
[7] Feldman, J., Cortell, R., and Gellhorn, E., *Am. J. Physiol.*, 1940, **131**, 281.

to us of interest to determine whether cocaine and bulbocapnine act similarly.

Methods. Rats weighing 200-250 g were used. Four groups of rats were studied. The first group comprised the control, in the second the medulla of the adrenals had been removed, in the third the vagi had been cut below the diaphragm, in the fourth these operations had been combined. Adrenalectomized and adrenalectomized-vagotomized rats received a 2% solution of NaCl instead of drinking water. All animals were fasted 18 hours. Blood sugar was determined by the Somogyi modification of the Shaffer-Hartman method. Fifty mg/kg of cocaine hydrochloride was injected subcutaneously in 6 rats in each of the above series. Blood sugar was determined prior to the injection and at hourly intervals over a period of 5 hours. The animals exhibited no marked symptoms with this dose, only a mild degree of excitement being observed in about one-fourth of the animals.

With bulbocapnine 2 experimental procedures were used. In the first, the effect of subcutaneous injection of 50 mg/kg of bulbocapnine phosphate (Merck) was studied in 7 normal, 6 adrenalectomized and 6 adrenalectomized-vagotomized rats over a period of 2 hours, blood sugar being determined at half hour intervals. In the second, the effect of the same dose of bulbocapnine phosphate was studied on 6 normal and 6 vagotomized rats over a period of 4 hours, blood sugar being determined at hourly intervals. In all cases, the animals showed akinesis and catalepsy, maintaining abnormal positions and hanging from a bar for periods of 1 to 3 minutes. Akinesis developed within 10 minutes after injection and lasted for approximately 1 hour.

Results. With cocaine hydrochloride, normal rats show a rise in blood sugar from a mean of 74.1 mg % to a maximum of 89.0 mg % at the end of 3 hours. By the end of 5 hours this has returned to a mean value of 79.2 mg %. With adrenalectomized rats, on the other hand, one observes a fall in blood sugar from a mean of 64.5 mg % to a minimum of 46.7 mg % at the end of 3 hours and a return to 54.1 mg % at the end of 5 hours. These results indicate that cocaine acts similarly to anoxia and to metrazol in stimulating the vago-insulin as well as the sympathetico-adrenal systems. If this hypothesis is correct, sectioning of the vagi in the adrenalectomized rats should, by elimination of the secretion of insulin *via* the vagi, eliminate the hypoglycemia. This is actually the case, cocaine having no significant effect on the blood sugar of adrenalectomized-vagotomized rats. Moreover, when the vagi are sectioned subdia-

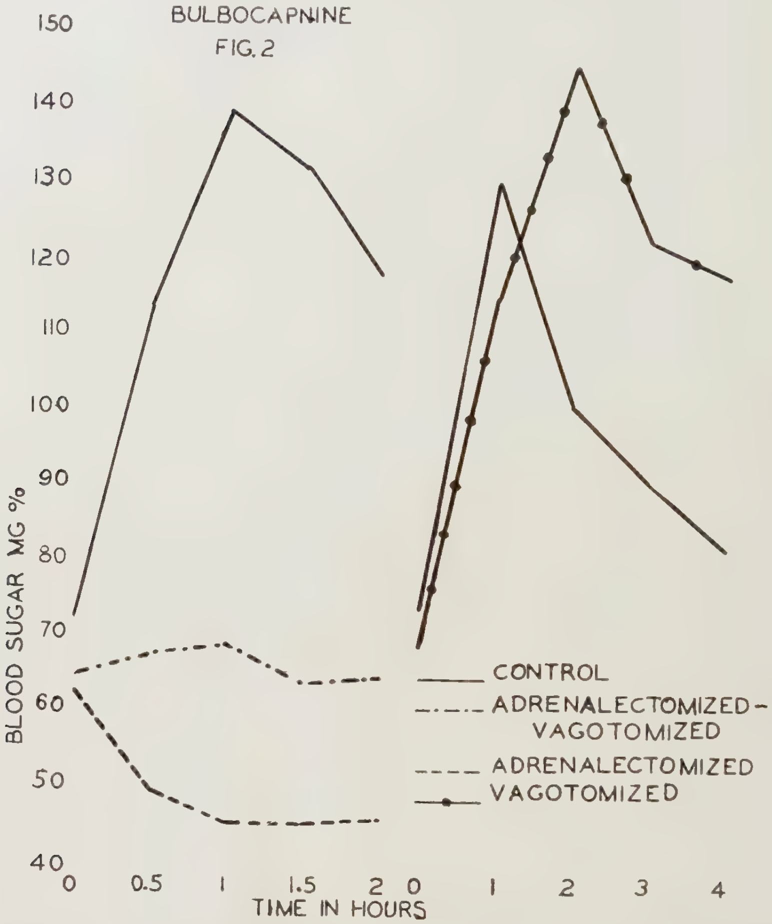


Influence of 50 mg/kg cocaine hydrochloride on the blood sugar of rats. Cocaine injected subcutaneously immediately after the first blood sample was taken. ———Control; - - - -adrenalectomized; -.-.-adrenalectomized-vagotomized; —●—●—vagotomized.

phragmatically in normal rats, one observes a greater hyperglycemic response than in control animals (Fig. 1). This we attribute to the elimination of the vagally controlled insulin secretion.

With bulbocapnine phosphate similar results are obtained (Fig. 2). Here again the normal animals show an increase in blood sugar even greater than with cocaine hydrochloride, the adrenalectomized a fall and the adrenalectomized-vagotomized no significant change. With the vagotomized animals, the rise in sugar is not as rapid as in the controls, but the maximum is higher and the effect much more prolonged. It seems safe, therefore, to conclude that here again we have a stimulation of both sympathetic and parasympathetic centers, and that the reaction of each of these centers may be demonstrated by selection of the proper conditions.

Summary. 1. Subcutaneous injection of cocaine hydrochloride causes hyperglycemia in normal rats and hypoglycemia in adrenalectomized rats. If the vagi are sectioned subdiaphragmatically in the adrenalectomized rats, there is no significant change in blood sugar. If the vagi are cut subdiaphragmatically in normal animals, there is a greater increase in blood sugar than in animals with intact vagi. 2. Similar results are obtained with bulbocapnine phosphate. 3. These results are interpreted to mean that cocaine and bulbocapnine although



Influence of 50 mg/kg bulbocapnine phosphate on blood sugar of rats. Bulbocapnine was injected immediately after the first blood sample was taken. Designation as in Fig. 1.

acting quite differently on the somatic nervous system have a similar action on the autonomic system. They stimulate both the vago-insulin and the sympathetico-adrenal system. The effect on the latter predominates in the normal animal.

11922

Zoological Specificity of the Tumoral Reaction Towards Estrogens in Rats.*

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While abdominal fibroids can be easily elicited in guinea pigs by a prolonged treatment with estrogens, they have never been reported in rats, mice and monkeys under similar experimental conditions. Two different possibilities may account for this zoological specificity of the tumoral reaction: first, the conjunctive tumorigenic threshold may be different according to the species; secondly, the reacting cellular type of the mesenchyme may be absent in certain species. Our quantitative experiments with abdominal fibroids in the guinea pig[1] offer the opportunity to study these two possibilities. We have, therefore, investigated the possibility of eliciting abdominal fibroids in rats by injecting a highly potent tumorigenic ester of estradiol in quantities many times those necessary to induce these tumors in the guinea pig.

Eleven castrate female rats (103 to 260 g) received thrice weekly subcutaneous injections of 10 γ of α-estradiol given as the 17-caprylic acid ester (14.6 γ of the ester); 7 castrate (60 to 295 g) and 9 non-castrate (65 to 247 g) females received 80 γ per injection. The animals were autopsied 90 to 100 days after the first injection. Uterine weights ranged between 160 and 1200 mg. Atypical proliferation of uterine epithelium with thickening, cornification and sometimes of precancerous developments were found even with 10 γ per injection (Fig. 1). Hypophyseal hypertrophy also was considerable (Fig. 2). Hypophyseal weights of 17 to 44 mg were reached with 10 γ per injection and of 20 to 192 mg with 80 γ per injection. The average hypophyseal weight per 100 g body weight was 14 mg in the group receiving 10 γ per injection, and 33 mg in those receiving 80 γ per injection, as against 4 mg per 100 g body

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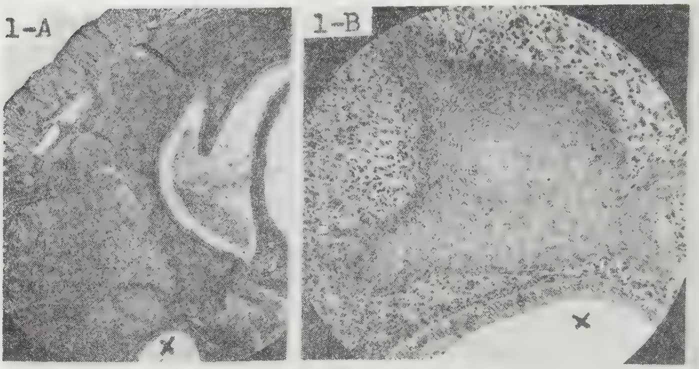
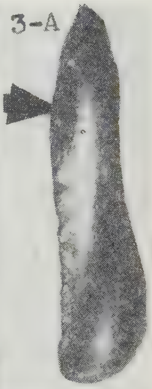
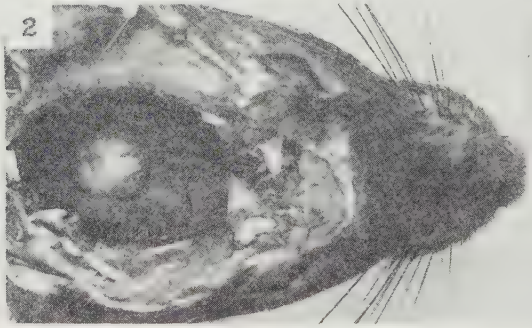
* This investigation has been aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research and Mr. Adolfo Eastman of Limache (Chile). The esterified estrogens were generously supplied by Dr. Carl Miescher of Ciba Pharmaceutical Products, Inc.

[1] Lipschütz, A., Vargas, L., Jr., Baeza-Rosales, H., and Baeza-Herrera, H., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 76.







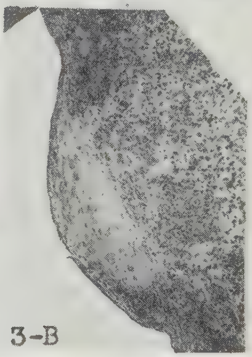


FIG. 1. Uterine horn of castrate rat (220 g) which received 38 injections of 10 γ of estradiol (in form of the 17-caprylate) in the course of 91 days. A. × 23. Cystic hyperplasia with beginning pyometra. Epithelial ingrowths into the submucosa. Note cornification in the lower right of the photograph. B. × 100. Part of the epithelial ingrowth, to show concentric disposition of cells; beginning of pearl formation in the center (XIV, R. 8).

FIG. 2. × 1.5. Enormous hypertrophy of the anterior lobe of the hypophysis in female rat, 38 injections of 80 γ, 91 days. Weight of animal 120 g; of hypophysis—66 mg (XIV, R. 23).

FIG. 3. Spleen of male rat (280 g), 28 injections of 80 γ, 62 days. A. × 1.5. Excrescences of whitish color on the surface. B. × 100. Fibrous structure of splenic excrescence (XIV, D. 24).

weight in the normal or castrate female.[2] Body weight which first increased, decreased later in all animals.[†] *None of the 27 females showed uterine or extragenital fibroids or fibrous strands.*

The quantity of the monocaprylate given in the above experiments was 5 to 40 times that necessary to elicit fibroids in the guinea pig (10 to 80 γ, compared with 2 γ). When calculated per 100 g of body weight the quantities injected in the rat were from 15 to 120 times that used in the guinea pig. As seen from the above statements various severe effects (decrease of body weight, atypical growth of uterine epithelium, and great increase in size of hypophysis) were produced in the rat but no fibroids were elicited.

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[2] Lipschütz, A., *Arch. Portugu. Soc. Biol.*, 1936, **5**, 179; Lipschütz, A., and Villagrán, G., *C. R. Soc. Biol.* (Paris), 1936, **131**, 203.

[†] The average weight of hypophysis per 100 g body weight has been calculated according to maximal body weights reached.

Similar experiments were made with 30 non-castrate males (33 to 330 g) which received 2 to 80 γ per injection thrice weekly in the course of 81 to 102 days. Hypophyseal weights 3 times that of normal males were obtained with 2 to 4 γ per injection (15 to 35 mg); the average hypophyseal weight per 100 g body weight was 8 mg compared with 2.5 mg in the normal male.[3] The corresponding averages with 8 to 10 γ per injection were 15 mg, and 34 mg with 80 γ. In the latter group there were hypophyseal weights of 110 to 140 mg in 4 out of 10 males. *No fibroids or fibrous strands were found.* On the contrary, with 80 γ of esterified estradiol and especially of the monocaprylate most male guinea pigs show fibrous strands and sometimes also fibroids.[3]

One may be inclined to conclude from these results that in the rat the cellular substratum which would be able to react towards estrogens with development of fibroids is lacking. However, there is one observation which seems contrary to this.

In a single male which died after having been treated for only 62 days (80 γ per injection) small white excrescences were found on the surface of the spleen (Fig. 3). Their microscopical structure was fibrous and similar, though not quite identical, to that of the "tumoral seed" of the guinea pig treated with estrogens.[4, 1] Though the tumoral seed cannot be as yet identified with fibroids, extensive work of Lipschütz and associates with guinea pigs has shown beyond any doubt that these small excrescences are to be considered as the sign of a beginning tumorigenic action[5] especially in the male guinea pig which has a lesser tumoral sensitivity than the female. The occurrence of similar structures in the rat, though in only one out of 58 animals treated, would be in favor of the idea that the difference between guinea pig and rat is only a question of threshold.

Whether or not this explanation is justified cannot as yet be said with certainty. It is not likely that differences of threshold can account for the differential behavior of the uterine mucosa and of the anterior lobe of the hypophysis in guinea pig and rat. Though atypical proliferation of the endometrium starts in the guinea pig even with minute quantities of esterified estradiol[6] one never observes cornification in this species even when great quantities are given for 10 months or more (unpublished results). On the contrary, in the rat cornification of the uterine mucosa occurs under these experimental conditions. Again, we never observed great hypophyseal hypertrophy in the guinea pig treated with estrogens (unpublished results of Lipschütz and Vargas). However, as shown by the present experiments, enormous hypophyseal enlargement in the rat, described by former workers as tumoral,[7] can be elicited with total amounts ranging from less than 0.4 to 3.0 mg of estradiol when given as the monocaprylate (40 injections of 10 to 80 γ).

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[3] Koref, O., Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol* (Paris), 1939, **130**, 303; Jedlicky, A., Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol.*, (Paris), 1939, **130**, 1464.

[4] Lipschütz, A., Iglesias, R., and Vargas, L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 788.

[5] Vargas, L., Jr., and Lipschütz, A., *C. R. Soc. Biol.* (Paris), 1938, **129**, 810.

Summary. A quantity of the monocaprylate of α-estradiol up to 40 times that which is able to induce abdominal fibroids in the *guinea pig,* is insufficient to elicit similar tumors in the *rat.* The same or smaller quantity induces in the rat a highly pronounced atypical proliferation of epithelial tissues (endometrium, anterior lobe of the hypophysis).

11923

Comparative Conjunctive Tumorigenic Action of Three Different Esters of Estradiol.*

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Our previous work has shown that the conjunctive tumorigenic action of estradiol in the guinea pig is greatly enhanced by esterification.[1] Two hundred γ of free estradiol[2, 3] are less active than 5 γ of estradiol given as monobenzoate.[4] The monocaprylic ester of

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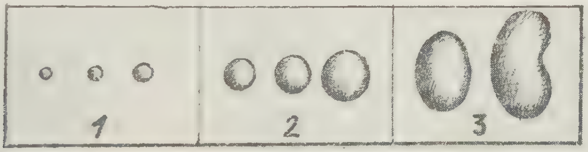
[6] Lipschütz, A., Vargas, L., Jr., Jedlicky, A., and Bellolio, P., *Am. J. Cancer,* 1940, **39**, 185.

[7] McEuen, C. S., Selye, H., and Collip, J. B., *Lancet,* 1936, **1**, 775; Zondek, B., *Lancet,* 1936, **1**, 776.

* This investigation has been aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research, and Mr. Adolfo Eastman of Limache (Chile). The esterified estrogens were generously supplied by Dr. Carl Miescher of Ciba Pharmaceutical Co.

estradiol was shown in our first set of experiments to be even more active than the monobenzoic one.[5] The results of a more extensive comparative study of the different esters are given in this paper.

As seen from Table I most animals show some fibrous reaction even with 5 γ per injection; there may be fibrous strands in the mesentery and a tumoral seed on the spleen, the stomach and the parts of abdominal wall surrounding the spleen. However, there is only a small number of animals with individual tumors of a certain size. We applied in our experiments a system of classification[6] which greatly facilitated our work, though the system is a rather arbitrary one (Figs. 1 and 2). The *total tumoral effect* represents the sum of 4 numerical values relating to the size of tumors of the uterus (subserous, mesometrial), "apical" tumors, tumors of the



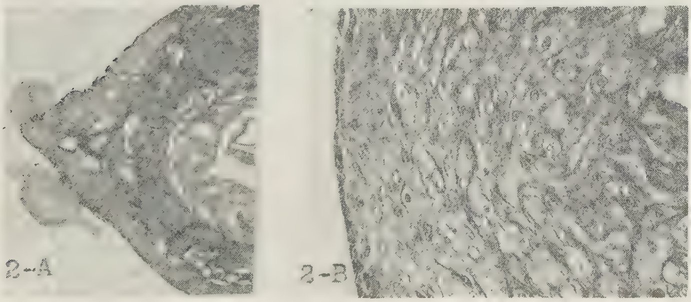


FIG. 1. Diagram of classification of tumors according to size. *Natural size.*

FIG. 2. Two subserous uterine fibroids induced by 40 injections of 5 γ of monobenzoate in the course of 3 months (VI. 96). A. × 10. Size of individual tumors somewhat less than class 1. Fibroids at both sides of ventral ridge of myometrium. Adenomatous polyps in the uterine cavity. B. × 200. Spindle shaped cells at the periphery; superficial cells flattened. Sclerotization in the depth of the fibroid.—Van Gieson.

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[1] Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol.* (Paris), 1939, **130**, 9.

[2] Lipschütz, A., Rodríguez, A., and Vargas, L., Jr., *C. R. Soc. Biol.* (Paris), 1939, **130**, 939.

[3] Rodríguez, F., *Public. Med. Exp.* (Chile), 1940.

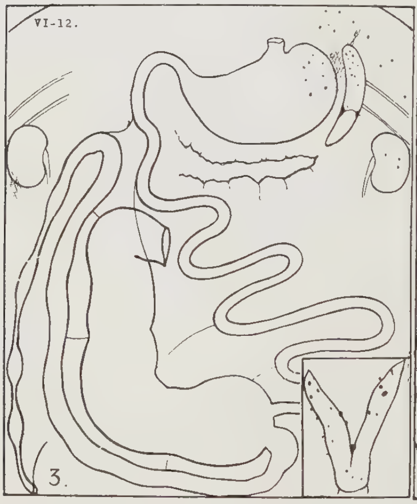
[4] Bellolio, P., *Public. Med. Exp.* (Chile), 1939.

[5] Jedlicky, A., Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol.* (Paris), 1939, **130**, 1466. The 17 benzoate-3-n-butyrate of estradiol also proved to be tumorigenic but the number of experiments was too small to allow quantitative interpretation.

[6] Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol.* (Paris), 1939, **131**, 27.

digestive tract and the abdominal wall, and splenic tumors (Figs. 3 and 4). The tumors of each of these 4 regions are arranged in 4 classes characterized by the values 0.5, 1, 2 and 3. The size of tumors of the last 3 classes is as indicated in Fig. 1. With 5 γ of estradiol per injection given as the monobenzoic or dipropionic ester only 8 out of 28 animals showed tumors of size 1 or over. The average total effect was 1. In each of these 2 lots there was only one animal with a total effect of 3 or 3.5 (Fig. 3). On the other hand, with the same quantity of estradiol given as the monocaprylic ester, 3 out of 4 animals showed a considerable tumoral reaction and the average total effect was 5, instead of 1 with the 2 other esters. Similar comparative results were obtained with 10 γ of esterified estradiol per injection (Table I).

We tried to explain the greater tumorigenic action of the monobenzoic ester as compared with that of free estradiol by the assumption[1] that by injecting the ester, which is slowly absorbed, thrice weekly a stable folliculinaemia might be maintained more easily than by injecting the free hormone which is rapidly absorbed and



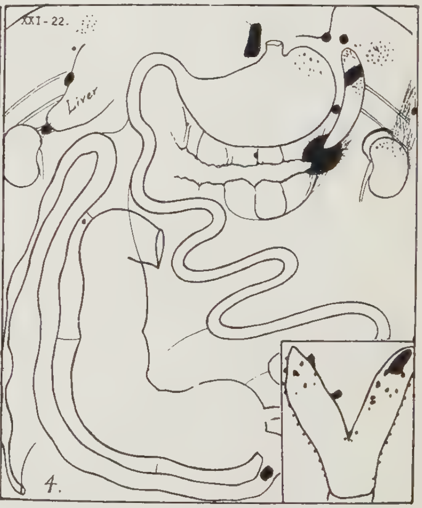


FIG. 3. Maximal reaction in animal receiving 40 injections of 5 γ of dipropionate in the course of 3 months (VI. 12). Multiple small uterine fibroids, subserous and mesometrial (class 2, due to their multiplicity). Tumoral seed on the stomach, abdominal wall, left kidney (class 0.5). Tumoral seed and small tumors on the spleen (somewhat less than class 1). Fibrous strands around spleen and right kidney. Total tumoral effect 3.5. × 0.4.

FIG. 4. Maximal reaction in animal receiving 39 injections of 5 γ of monocaprylate in the course of 3 months (XXI. 22). Multiple uterine fibroids, subserous and mesometrial (class 3). Various tumors of different size of the digestive tract, liver and abdominal wall (class 3) and of the spleen (class 3). Tumoral seed on spleen, stomach, abdominal wall and left kidney. Cyst in thickened capsule of left kidney. Fibrous strands around upper pole. Total tumoral effect 9. × 0.4.

TABLE I.
Seventy Castrate Female Guinea Pigs, Treated with Subcutaneous Injections of Various Esters of Estradiol During 3 Months.

Ester	Quantity estradiol* inj. thrice weekly	No. of inj.	Total animals	No. with some conjunctive reaction	No. with tumors, class 1 or larger	Avg total tumoral effect	No. of animals with total tumoral effect of 3 or over
Monobenzoic	γ 5	40	14	14	2	*1*	1
Dipropionic	5	40	14	14	6	*1*	1
Monocaprylic	5	40	4	4	3	*5*	3
Monobenzoic	10	40	15	12	4	*1*	0
Dipropionic	10	40	14	14	4	*1*	1
Monocaprylic	10	39	9	9	9	*6*	5

*The equivalent of estradiol is given, and not the weight of the esterified substance.

destroyed. Experiments with tablets of free estradiol introduced under the skin by which a continuous flow of hormone in the body is maintained[7] favor our assumption. But why then the difference in the tumorigenic action of the various esters? It has been shown by Miescher, Scholz and Tschopp[8] that when the total dose is given in 2 injections on 2 consecutive days, certain esters induce in the rat a greater and more lasting augmentation of the uterus than others. One may assume that in the work of Miescher, *et al.,* the prolonged action of certain esters is responsible for their greater hysterotrophic effect. However, in our work with tumorigenesis in which the different estrogens are injected thrice weekly in the course of several months, this explanation seems insufficient. One may tentatively suggest that esterification is also a means of protecting the natural estrogen against inactivation and that the degree of protection differs according to the acid used. This suggestion is indeed speculative and needs experimental demonstration.

Summary When 40 subcutaneous injections of 5 to 10 γ of an esterified estradiol (monobenzoic, dipropionic or monocaprylic ester) or a total of 200 to 400 γ are given to castrate female guinea pigs in the course of 3 months, uterine and extrauterine fibroids will appear. The number of animals in which fibroids are induced is larger with the monocaprylic ester; the size of the fibroids also is much more remarkable with the monocaprylate.

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[7] Lipschütz, A., and Vargas, L., Jr., *Lancet,* 1939, **1**, 1313.

[8] Miescher, K., Scholz, C., and Tschopp, E., *Biochem. J.,* 1938, **32**, 725.

11924

Influence of Bile Acids, Vitamin K and Cincophen on Erosions of the Chick Gizzard Lining.

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We have reported[1, 2] the curative and preventive effect of bile acids, particularly cholic acid, in experimental deficiency lesions or erosions of the chick gizzard lining. These studies have been continued.

The basal, gizzard-factor-deficient diet employed, the care of chicks, and the methods of analysis for cholic acid and of scoring gizzard erosions were the same as previously described.[2] Chicks were deprived of food for 12 to 14 hours previous to killing since this practice causes a greater accumulation of bile in the gall bladder.

The results of further experiments (Table I) confirm our earlier report.[2] Cholic acid and dehydrocholic acid consistently show strong preventive action against gizzard erosions at ½% of the diet. Dehydrocholic acid, the only bile acid sufficiently non-toxic to be administered by injection, is not preventive when so given. Deoxycholic acid is undoubtedly the least effective of these bile acids.

Since our first reports, certain workers[3, 4] have suggested that the

TABLE I.
Effect of Bile Acids on Chick Gizzard Erosion.

Supplement to basal diet	Level in diet, %	No. of groups†	Avg erosion score divided by basal group erosion score	Avg cholic acid content of dried gall bladder bile‡ mg per g
None	—	15	1.00	111
Cholic acid	0.50	9	0.15	542
,, ,,	0.25	2	0.36	540
Dehydrocholic acid	0.50	5	0.16	104
,, ,,	0.50*	2	0.92	86
Deoxycholic acid	0.50	5	0.73	85
Glycocholic acid	0.50	1	0.58	393

*Administered by frequent intramuscular injection of the aqueous sodium salt in amounts equivalent to 0.50% of the diet consumed.

†Each group contained 7 or more chicks, in most cases 10.

‡Cholic acid was determined on pooled samples from each group.

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[1] Almquist, H. J., *Science*, 1938, **87**, 538.

[2] Almquist, H. J., and Mecchi, E., *J. Biol. Chem.*, 1938, **126**, 407.

[3] Lansing, A. I., and Miller, D., *Poultry Science*, 1940, **19**, 258.

[4] Cheney, G., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 190.

mechanism of the effect of the bile acids is that of promoting the absorption of a fat-soluble, anti-erosion factor. A closer consideration of our results would have made it apparent that such explanation is unlikely, since deoxycholic acid is particularly known to facilitate absorption of the fat-soluble vitamins,[5] yet it is comparatively inactive against gizzard erosions.

The gall bladder bile analyses show that the anti-erosion activity is not merely a process of increasing the cholic acid in the bile. One-fourth percent of cholic acid causes the same cholic acid content per gram of dried bile but is less effective than ½% cholic acid in preventing erosions. It would appear in this case, as in the experiments with injected dehydrocholic acid, that the concentration of bile acid coming in direct contact with the lining is important. Dehydrocholic acid does not raise the bile cholic acid above that of the basal groups, yet has a strong preventive effect. This bile acid evidently has a specific action like that of cholic acid. That dehydrocholic acid is not, to any significant extent, converted to cholic acid is indicated by the cholic acid content of the gall bladder bile which is even lower than that of the basal groups.

Certain writers[6, 7, 8] have differentiated between kinds of gizzard lining abnormalities such as roughening and thickening, sub-surface hemorrhagic lesions, crater-like lesions and necrotic areas. All these we have observed in our chicks also. It is a question whether these conditions are really differences in kind or merely in degree. The strongest evidence for the latter view, that all the abnormalities are but manifestations of one primary deficiency, lies in the observation that one pure, natural substance, cholic acid, is capable of preventing all these conditions.

In spite of published evidence[9] that gizzard erosions are independent of vitamin K deficiency, a relation has again been suggested,[3] without supporting data. We had noted that prolonged massive doses of the highly potent antihemorrhagic compound, 2-methyl-1,4-naphthoquinone, tended to reduce the discoloration due to decomposed blood around the erosions, but not the actual erosions. The more potent, water-soluble, 2-methyl-1,4-naphthohydroquinone

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[5] Cohn, E. T., and Schmidt, C. L. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 443; Schmidt, C. L. A., *Pac. Coast Med.*, 1938, **5**, 7.

[6] Lansing, A. I., Miller, D., and Titus, H. W., *Poultry Science*, 1939, **18**, 475.

[7] Blount, W. P., *Veterinary J.*, 1939, **95**, 301.

[8] Bird, H. R., Oleson, J. J., Elvehjem, C. A., Hart, E. B., and Halpin, J. G., *Poultry Science*, 1937, **16**, 238.

[9] Almquist, H. J., and Stokstad, E. L. R., *Nature*, 1936, **137**, 581; *J. Nutrition*, 1937, **13**, 339.

TABLE II.
Effect of Various Forms of Vitamin K on Gizzard Erosions.

Supplement to basal diet	Level in diet, mg per kg	Avg erosion score divided by basal group erosion score	Avg prothrombin clotting time, sec.
Vitamin K_1*	100	0.82	28.9
,, ,, *	½ }	0.18	34.0
Cholic acid	5000 }		
2-methyl-1,4-naphthohydroquinone diphosphoric acid†	50	1.27	
Phthiocol‡	2,500	0.90	
Practical chick mash	total diet	0.00	28.1

*No alfalfa extract present in basal diet in these cases.

†Hydrated tetra sodium salt. Fed at approximately 500 times the required level for normal prothrombin clotting time as determined by previous assay.

‡Fed at approximately 100 times the required level for normal prothrombin clotting time as determined by previous assay.

diphosphoric acid ester and the synthetic vitamin K_1, 2-methyl-3-phytyl-1,4-naphthoquinone, were fed in large doses, but had no effect in reducing gizzard erosion. In the presence of a sub-optimal amount of vitamin K_1 (½ mg per kg of diet) cholic acid was able to maintain the erosion score at a low value (Table II). We have repeatedly measured blood clotting times and blood prothrombin levels of groups of chicks that proved to have severe gizzard erosions, finding these blood characteristics quite normal. It would be hard to obtain more conclusive evidence of a fundamental difference in the two deficiencies.

A large number of substances have been tested and found ineffective. These include cholesterol, phytosterol, yeast sterols, α-tocopherol, phytol, glucuronic acid, gum arabic, agar, choline, creatine, phytic acid, aloin, mucin powder, linoleic acid, dried stomach lining, butter, artichoke, avocado, papaya, garlic, whole-milk powder, and copper sulphate. Chondroitin and certain samples of wheat germ oil showed a slight protective effect at levels of 5% of the diet. Hog bile salts which contain relatively little cholic acid proved less effective than beef bile salts which are a good source of cholic acid.

It is well known that the compound cincophen, administered orally or intravenously, causes gastric ulcers in the dog.[10] Recently it has been reported that cincophen also produces lesions or erosions of the chick gizzard lining.[4] Preliminary tests showed that the addition of one-half percent of cincophen to our basal deficiency diet caused a slight increase in severity of the lesions. The lesions remained, superficially at least, the same as those usually observed, with the exception of an occasional instance of cylindrical penetration

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[10] Bradley, W. B., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 143.

TABLE III.
Effects of Additions of Cincophen and of Bile Acids to a Practical Chick Ration.

Supplement to practical ration	Level in diet, %	Avg erosion score*	Gall bladder bile† Vol. per chick, cc	Gall bladder bile† Cholic acid in dried bile, mg per g
None	—	0.10	0.19	91
Cincophen	1.0	0.93	0.20	113
Cincophen Cholic acid	1.0 1.0	0.13	0.67	478
Cincophen Ox bile salts	1.0 2.0	0.36	1.16	413

*Average erosion score for 15 chicks.
†Pooled samples from each group.

into the underlying tissues and, in a few cases, perforation. Cholic and dehydrocholic acids were found to exert a protective effect even in the presence of cincophen.

Chicks reared on a practical mash which ordinarily permitted no erosions were found to develop erosions closely resembling the deficiency type, even to the usual discoloration, when fed cincophen. At least 1% in the diet was required to cause severe erosions. Two percent of cincophen was decidedly inhibitory to growth and caused heavy mortality. Experiments were continued with the practical mash as it was felt that the lesions produced could be attributed entirely to cincophen. Some results are given in Table III.

Development of the cincophen-induced erosions was largely prevented by the simultaneous feeding of cholic acid and of beef bile salts. In overcoming the effects of cincophen on the gizzard lining, cholic acid evidently functions in a similar way as in the prevention of deficiency erosions. The fundamental rôle of cholic acid in the maintenance of the lining is, therefore, further emphasized.

It is of interest to mention reported indications that cincophen tends to reduce cholic acid synthesis in the dog.[10] The analyses of the chick gall bladder bile do not, however, give any indication of a reduction of amount of cholic acid present in the chick when cincophen is fed as compared to no cincophen. On the other hand, the increased bile volume and cholic acid content of bile in the remaining groups may have been assisting factors in the reduction of the erosion score.

The above evidence is in accord with our previously expressed view,[2] based on the presence of bile pigment and of cholic acid in combination with the lining, that bile and especially cholic acid, coming in periodic direct content with the lining serves naturally to aid in maintaining the lining in sound condition.

We are indebted to Riedel de Haen, Inc., for some of the bile acid preparations used, and to Dr. David Klein, the Wilson Laboratories, Chicago, for preparations of bile salts, chondroitin, mucin and stomach lining.

11925

Effect of Adrenalectomy on Intestinal Absorption Involving Osmotic Work in Rats.

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The abnormalities of the mineral metabolism of adrenalectomized animals are common knowledge, and have been the subject of several comprehensive reviews (*e. g.*, Kendall[1]). In the kidney it has been possible to study the course of interference with the normal sodium and potassium balance in some detail. Further analyses in the kidney are however confronted with great difficulties. Fortunately, disturbances of reabsorption by the kidney tubuli can often be demonstrated by analogous experiments with the small intestine. Phloridzin interference with sugar reabsorption in proximal parts of the tubuli can be seen for example in the inhibitory effect of phloridzin on active sugar absorption in the intestine (Wertheimer[2]). Visscher and coworkers[3] in a series of investigations have shown that an active transport of ions in a direction opposite to that of the concentration gradient can regularly be demonstrated in the small intestine under specified conditions. This finding seems to be of general physiological significance; a similar transport of ions against the direction of the gradient has been shown to exist in plant roots by Lundegardh[4] and in several freshwater animals by Krogh.[5] In this connection mention may also be made of the papers of Steward[6] and Keys.[7] It seemed of some importance in view of these results to ascertain whether an active transport of ions against the direction of the gradient could be demonstrated also in the small intestine of adrenalectomized animals. Fifty-four animal experiments testing this question have been carried out by us. Our earlier experiments were carried out according to the procedure used by Wilbrand and Laszt[8] for the study of active sugar absorption in the rat intestine.

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[1] Kendall, E. Z., Congress Bericht 1, 16 Intern. Physiol. Congr., August, 1938, Zurich, p. 47.

[2] Wertheimer, E. *Pflüger's Arch.*, 1933, **233**, 514.

[3] Ingraham, R. C., and Visscher, M. B., *Am. J. Physiol.*, 1938, **121**, 771.

[4] Lundegardh, H., *Biochem. Z.*, 1937, **290**, 104.

[5] Krogh, A., *Scand. Arch. Physiol.*, 1937, **76**, 60.

[6] Steward, F. C., *Trans. Farraday Soc.*, 1937, **33**, 1006.

[7] Keys, A., *Trans. Farraday Soc.*, 1937, **33**, 930, 972.

Considerable differences in total sodium and chlorine absorption between normal and adrenalectomized rats were found. (In 12 experiments absorption of NaCl in adrenalectomized rats during a time interval of 40-80 min. was nil, whereas in normal rats absorption during the same interval varied in extent from 20 to 80%.) Ionic migration against the gradient, as shown by Visscher, was, however, difficult to demonstrate by this method. Active sugar absorption on the other hand, was clearly evident. This finding shows that active ion absorption by the small intestine is distinct from active sugar absorption, being of a far greater lability and to be ascribed to a distinct energetic process.

In our later experiments the method of Visscher and his coworkers[9] was applied. This differs from Laszt's method only in that for cleaning, gentle stroking of the intestine is replaced by rinsing. With this technic results obtained using normal rats were in full accord with the findings of Visscher.

The adrenalectomized rats were maintained on a diet relatively rich in common salt but poor in potassium. Rubin-Krick solution was given to drink. In addition, the animals received injections of NaCl and sodium bicarbonate solution, as in the experiments of Laszt.[10] All animals used were in good health. They were maintained throughout the course of the absorption experiments on an electrically heated table at 38°C. The external behavior of adrenalectomized and normal rats was similar. Shock phenomena (*e. g.,* temperature drop) were not observed. Typical experiments are shown in Table I.

The experiments show that NaCl resorption in the small intestine of adrenalectomized, but otherwise healthy, rats is markedly retarded and that in such rats resorption against the direction of the concentration gradient is no longer possible. The inability of adrenalectomized rats to effect an active ion transport is already evident two days after operation. By administration of desoxycorticosterone, on the other hand, normal resorption of Cl may be reëstablished. Rats maintained on vitamin B-free diet do not suffer from interference with the normal active resorption of ions despite marked

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[8] Wilbrand, W., and Laszt, L., *Biochem. Z.*, 1933, **259**, 398.

[9] Lifson, N., *Am. J. Physiol.*, 1940, **128**, 607.

[10] Laszt, L., *Nature,* 1939, **144**, 244.

TABLE I.
Absorption of NaCl-Na_2SO_4 in Small Intestine Following the Method of Visscher.

		Volume, cc		Chloride, mM/L			Sodium, mM/L			
Body wt, g	Time, min.	Begin	End	Intro.	Found	% Abs.	Intro.	Found	% Abs.	Notes
170	80	5.0	0.7	78.5	33.0	94.0	191.0	243.0	81	(1)
141	80	4.0	1.0	78.5	33.2	90.0	191.0	191.0	76	(2)
140	40	4.0	1.0	78.5	66.3	79.0	191.0	184.0	70	(3)
181	80	5.0	5.0	78.5	107.8	—	191.0	159.0	20	(4)
193	80	5.0	3.9	78.5	91.3	10.6	191.0	174.0	41	(5)
187	80	5.0	1.4	78.5	45.2	81.0	191.0	143.6	80	(6)
158	70	4.0	2.1	78.5	79.0	47.4	178.5	163.2	55	(7)

Notes:
(1) Semi-adrenalectomized.
(2) Maintained 4 weeks on vitamin B-free diet; loss in body weight 20 %.
(3) Thyroidin admin. for 14 days in daily dose of 10 mg.
(4) 20 days after adrenalectomy.
(5) 2 ’’ ’’ ’’
(6) 7 ’’ ’’ ’’ , 1 mg desoxycorticosterone 3 days before exp.
(7) Addition of 1/10,000 parts monoiodoacetate.

loss of weight. For the ultimate identification of the energy-yielding reaction it is of interest to note that a 1/10,000 solution of monoiodoacetate completely inhibited all ion resorption against the direction of the gradient. Thyreotoxic animals do not show clearcut deviations from normal in regard to resorption.

At this stage of our investigations, the study of Dennis and Wood[11] which reports experiments with adrenalectomized dogs in both the acute and chronic condition and yielded results similar to those obtained by us was published. We are, therefore, able to offer full confirmation of the results obtained by these authors.

11926 P

Production of Azoospermia in Man from the Use of α-Estradiol Benzoate (Progynon-B).*

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This preliminary report deals with the effect of α-estradiol benzoate upon spermatogenesis in a 72-year-old man, who has been

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[11] Dennis and Wood, *Am. J. Physiol.*, 1940, **129**, 182.

* α-Estradiol Benzoate was generously supplied by the Schering Corporation under the trade name of ‘‘progynon-B.’’

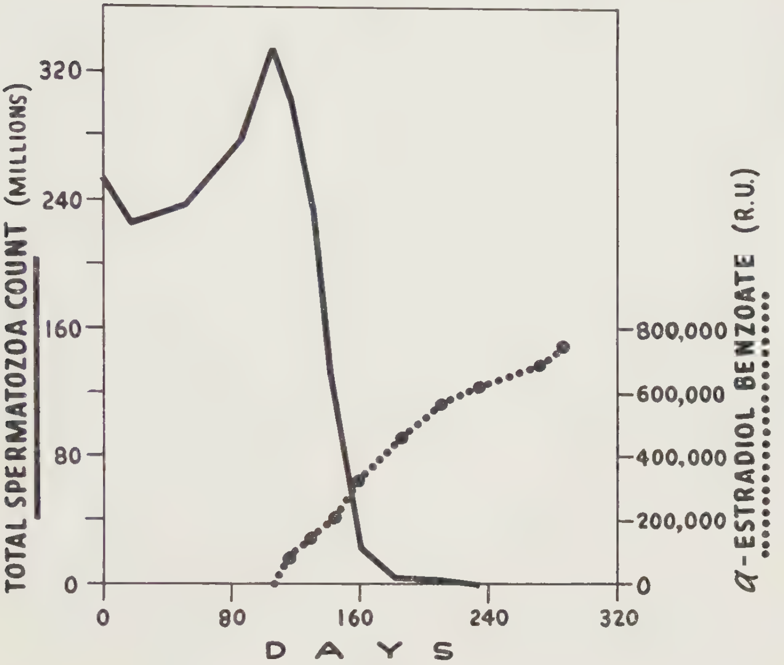

FIG. 1.

under observation since August, 1939, because of an enlargement of the prostate gland. He received subcutaneous injections of 6,000 to 10,000 rat units of this material 4 to 6 times a week for 26 weeks. The graph illustrates the effect upon the number of spermatozoa. Five spermatozoa counts were made over a period of 105 days before the injections of α-estradiol benzoate were started. After the patient had received a total of approximately 300,000 r.u. in 51 days a striking decrease in the number of spermatozoa occurred. As the treatment was continued, the number of spermatozoa further decreased until an azoospermia developed by the 2~~1~~29th day of treatment, when a total of 631,000 r.u. had been administered.

It is of interest to know that male sex hormone in the form of testosterone propionate when given in daily injections of 25 mg over a prolonged period of time will likewise produce an azoospermia in man.[1]

The determination of the number of spermatozoa in the artificially ejaculated specimens was obtained by counting them on a blood counting chamber, after diluting them in a white blood pipette with a solution of 5% soda and 1% formalin in water, calculating the number in cubic millimeters by the method used to determine a white blood count, multiplying the result by 1000 to obtain the number per cubic centimeter, and then multiplying the result by the number of cubic centimeters of semen.

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[1] Heckel, Norris J., *J. Urol.*, 1940, **43**, 286.

It has previously been observed that in the rat, atrophy of the testes is caused by prolonged injections of large doses of α-estradiol benzoate.[2] Also, in the brown leghorn cock, extreme atrophy of the testes and comb could be produced by prolonged administration of large doses of this material, but when the injections were discontinued, the comb growth took place and the testes increased in size.[3]

Summary. The subcutaneous injection of 6,000 to 10,000 r.u. of α-estradiol benzoate, 4 to 6 times weekly, for 26 weeks, produced an azoospermia in a 72-year-old man. The duration of treatment, the daily dosage, and the variation in the spermatozoa counts are presented in the graph.

11927 P

Cultural Characteristics of *Pityrosporum ovale*—A Lipophylic Fungus. Nutrient and Growth Requirements.

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In an earlier paper[1] the successful cultivation of *Pityrosporum ovale* was reported. The addition of fats to the basal medium, wort agar, was found necessary to obtain subcultures. Of the pure fatty acids tested oleic gave the best results. Oleic acid was used by Fleming[2] for cultivation of the acne bacillus and has since been used in a number of bacteriological media. Cohen and Mueller[3] have recently shown its importance for *C. diphtheriae*. However, the *Pityrosporum* is probably the first microörganism reported to need fats or fatty acids as an indispensable factor for growth. Consequently further study of its nutritional requirements seemed worthwhile.

A simple solution devised by Currie for the study of the citric acid fermentation of *Aspergillus niger* served as a basic medium with the exception that dextrose was substituted for sucrose. It had the following composition: NH_4NO_3 2-2.5 g, KH_2PO_4 0.75-1 g, $MgSO_4$ 0.2-0.25 g, dextrose 50 g, water 1000 cc. Fifty cubic centimeters were distributed in 150 cc Erlenmeyer flasks (or 100 in 250 cc flasks) and the substances to be tested added. A suspension of the fungus was washed in Currie's fluid or distilled water in the centrifuge and resuspended in fresh fluid. Two or 3 drops of a thick suspension was used as the inoculum.

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[2] Del Castillo, E. B., et Sammartino, R., *C. R. Soc. de Biol.*, 1938, **129**, 870.

[3] Emmens, C. W., *J. Physiol.*, 1939, **95**, 379.

[1] Benham, R. W., *J. Invest. Dermat.*, 1939, **2**, 187.

[2] Fleming, A., *Lancet*, 1909, 1035.

[3] Cohen, Sydney, and Mueller, J. Howard, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 244.

In such media with various supplements the *Pityrosporum* grows both in granules which settle to the bottom of the flask and as a film or scum covering the surface and spreading up the sides of the flask above the level of the medium. When oleic acid is present the earliest growth appears as fine particles in or attached to the globules of acid which float on the surface. Inability to obtain even suspensions which could be measured in a colorimeter has prevented the making of quantitative records of growth. Dustlike particles scattered through the medium are recorded as "faint growth," a heavy suspension of particles with a thin film on the surface as "good growth," and larger granules in the depth with a thick membrane on the surface and adhering to the sides of the flask as "heavy growth".

In the Currie solution no definite development occurs if the inoculum is well washed. A faint growth of dustlike particles usually appeared after 2 or 3 weeks in earlier experiments when unwashed cells were used for seeding.

The addition of oleic acid .01% permits a slow faint growth. Increasing the oleic acid up to 1% favors growth, but beyond this point no increase was observed. In a 1% solution faint growth is obtained in 4 weeks.

The addition of 0.5% Asparagin does not permit growth in plain Currie broth but increases the growth in the presence of oleic acid. Asparagin plus 1% oleic acid gives good growth in 1-2 weeks and heavy growth in 2-4 weeks.

On account of the demonstrated relationship of another unsaturated fatty acid, linoleic, and pyridoxin in rat nutrition[4, 5] it seemed of special interest to test the action of this constituent of Vitamin B. Pyridoxin accelerates growth in the presence of oleic acid with or without asparagin. The effect seems more evident when the oleic acid is reduced to .01%, which retards growth in the controls.

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[4] Buch, T. W., *J. Biol. Chem.*, 1938, **124**, 775.

[5] Gross, Paul, *J. Invest. Dermat.*, 1940, **3**, 505.

Thiamin has a more marked accelerating effect in similar combinations, but the effect is just as evident in the cultures containing 1% oleic acid as in those containing .01%.

With .01% oleic acid and 0.5% asparagin and thiamin, good growth is obtained in 5-10 days. At this time flasks without thiamin show only a trace, but after 4 weeks both show heavy and apparently equal growth. These effects were observed when as little as 1 gamma of the vitamin was added to 50 cc of medium. On the other hand, amounts of pyridoxin and thiamin up to 2.5 mg per 50 cc were without effect in the absence of oleic acid.

Conclusions. The *Pityrosporum ovale* grows in the presence of inorganic salts, glucose and oleic acid. The addition of asparagin accelerates growth and increases the final yield.

Thiamin and pyridoxin accelerate development but are not essential to the growth of this fungus under the conditions studied.

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Non-Availability of Gum Acacia as a Glycogenic Foodstuff in the Rat.

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No biochemical analyses have been made to test whether the gum acacia molecule passes entirely undigested through the gastrointestinal tract. Observations on poorly controlled feeding experiments, cited in the older literature,[1] are reported to have shown that gum acacia, without other dietary supplement, is a deficient foodstuff. Since gum acacia is a rather important physiological tool,[1] any information concerning its chemistry or physiological properties is of some interest.

The question arose whether all of the sugar components of the molecule[2] are tightly bound into a "main" chain molecule, or whether some of them are possibly attached as "side" chains to the main molecule. In the latter case, they might be vulnerable to the digestive enzymes, and thus become materials available for glycogenesis.

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[1] Amberson, W. R., *Biol. Rev.*, 1937, **12**, 48.

[2] Butler, C. L., and Cretcher, L. H., *J. Am. Chem. Soc.*, 1929, **51**, 1519.

Two groups of 15 young male rats (avg. wt. 140 g) were removed from a normal diet of dog mash, placed in individual cages, and fasted for 48 hours. Five individuals in each group were given 10 g of cacao butter, the remaining ten, 10 g of a mixture containing 34% of white powdered gum acacia (Arthur H. Thomas) and 66% of cacao butter. Tests for free reducing substances in the gum acacia were negative. At the end of 72 hours each rat was anesthetized with 0.5 cc of 10% sodium amytal administered intraperitoneally. The liver was immediately extirpated and placed in a tared 50 cc centrifuge tube containing 15 cc of 30% potassium hydroxide. Glycogen was obtained by means of Good's modification[3] of Pfluger's method. Glucose was determined upon an aliquot of the hydrolyzed glycogen according to the method of Shaffer and Hartman.[4]

The livers of the 2 control groups contained an average of 0.15% and 0.13% glycogen by weight, respectively, while those of the acacia-fed groups contained an average of 0.24% and 0.17% glycogen by weight, respectively. The variation in the percent of glycogen by weight between the livers of the individual rats in the control groups ranged from 0.03% to 0.29%, with a mean in each group of 0.12% and 0.17%, respectively. Variations in percent glycogen by weight in the livers of the acacia-fed groups ranged from 0.04% to 0.31% in the various individuals. These had a mean value for each group of 0.27% and 0.18%, respectively.

In previous papers reported by Krantz, *et al.*,[5, 6] it has been established that the assay of glycogenic activity on the part of a compound administered as it was in these experiments does not become significant unless the percentage increase has an average value of the order of 300% or more.

It is concluded that the difference in liver glycogen between the control and the acacia-fed rats is insignificant. No part of the gum acacia molecule is subject to disintegration by the enzymes of the digestive tract of the rat. Orally administered gum acacia is excreted unchanged in the feces.

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[3] Good, C. A., Kraemer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

[4] Shaffer, P. A., and Hartmann, A. F., *J. Biol. Chem.*, 1920, **45**, 349.

[5] Carr, C. J., Musser, R., Schmidt, J., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1933, **102**, 721.

[6] Krantz, J. C., Jr., Evans, W. C., and Carr, C. J., *Quart. J. Pharm. and Pharmacol.*, 1935, **8**, 213.

11929 P

Effect of Administration of Thyroid Extract and of α-Dinitrophenol upon Dark Adaptation.

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Previous studies[1, 2] have shown that the process of dark adaptation of the eye may be disturbed in certain patients having cirrhosis of the liver or renal stone. The abnormal dark adaptation was characterized by (a) elevation of the cone and rod thresholds, (b) reduction in the speed of the process, or (c) a combination of these two effects. Other studies[2, 3] have shown that simple Vitamin A deficiency, as from diets deficient in the vitamin or from obstructive jaundice, may produce threshold changes alone. However, the reduction in speed of adaptation appeared to be a different phenomenon. Since this latter change, like the elevated visual thesholds, responded favorably to Vitamin A therapy, the delay in adaptation was interpreted as possibly a manifestation of faulty utilization of the vitamin.

Attention, therefore, was directed to other factors which might influence the speed of dark adaptation and which thus might be involved in the intermediary metabolism of Vitamin A. The method and apparatus for measuring dark adaptation was similar to that previously employed.[2] Serum Vitamin A and carotenoids were determined by a modification of the method described by Kimble.[4] The usual hospital diet without Vitamin A concentrates was fed throughout the period of observations.

The oral administration of thyroid extract or of α-dinitrophenol to 3 patients with markedly prolonged dark adaptation produced sharp changes in both rod and cone functions. The effect of thyroid extract was tested 4 times in 3 patients, whereas the effect of α-dinitrophenol was tested 2 times in 1 of these patients.

Table I records values for dark adaptation and for serum Vitamin A and carotenoids in these 3 patients. Only the initial and final values for each test period are tabulated. The data show a marked increase in speed of adaptation and lowering of visual thresholds

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[1] Haig, C., Hecht, S., and Patek, A. J., Jr., *Science*, 1938, **87**, 534.

[2] Patek, A. J., Jr., and Haig, C., *J. Clin. Invest.*, 1939, **18**, 609.

[3] Hecht, S., and Mandelbaum, J., *Am. J. Physiol.*, 1940, **130**, 651.

[4] Kimble, M. S., *J. Lab. Clin. Med.*, 1939, **24**, 1055.

TABLE I.

Values for Dark Adaptation and for Serum Vitamin A and Carotenoids Before and After Administration of Thyroid Extract and of α-dinitrophenol.

Patient	Exp. No.	Days of thyroid (T) or α-dinitrophenol (D)	Daily dose, *gr.*	Dark adaptation thresholds at various periods in dark *Log $\mu\mu$ lamberts*			Cone/Rod transition point, *min.*	Serum Vit. A—I.U./100 ml.	Serum carot.—μg/100 ml.	B.M.R.
				2 min.	15 min.	40 min.				
E.R., 55 yrs, ♀ Rheumatoid arthritis. Renal stone removed 7 yrs ago.	1	0		**7.7***	6.9	4.7	15.0	105	56	— 2
		13 T	2	**6.3**	4.0	2.7	4.5	93	42	+12
J.C., 64 yrs, ♂ Cirrhosis of liver	2	0		**7.4**	7.0	4.9	14.5	122	116	— 9
		42 T	1	**7.0**	5.1	4.2	8.1	64	116	+12
	3	0		**7.6**	7.0	4.5	14.8	190	149	—11
		9 D	1.1	**6.8**	5.4	3.7	9.0	134	166	+ 9
	4	0		**7.2**	6.7	4.4	13.5	—	—	—11
		8 D	1.1	**6.9**	3.9	2.1	4.2	—	—	—10
J.S., 35 yrs, ♂ Cirrhosis of liver Myxedema	5	0		**8.1**	6.7	5.0	12.8	0	458	—34
		121 T	1-3	**6.5**	4.4	4.2	4.5	9	99	— 4
	6	0		**7.6**	5.6	4.8	9.8	70	200	—24
		56 T	1-3	**6.8**	4.7	4.2	5.5	56	158	—10

*Cone readings in bold face type.

after feeding either thyroid extract or α-dinitrophenol. The type of response is similar to that observed with Vitamin A therapy, but it is accelerated. For example, in previous experiments[1, 2] patient J. C. required 250,000 to 500,000 I.U. Vitamin A daily for 2 months before normal values for dark adaptation were attained. In the present study such values were reached in 3 to 10 days after feeding of either thyroid extract or α-dinitrophenol.

In 4 out of 5 experiments the serum Vitamin A level was lowered by therapy. In the exceptional case (Exp. 5) a rise in serum A occurred concomitantly with a striking reduction in serum carotenoids from abnormally high to normal levels. It is possible in this case that the conversion of carotene to Vitamin A was facilitated by thyroid extract. This possibility is suggested by the studies of Fasold and Heidemann,[5] and of Clausen and McCoord.[6]

The mechanism by which the feeding of thyroid extract or α-dinitrophenol accelerates the rate of dark adaptation and lowers the visual thresholds is not clear. The fact that both substances bring about the same response suggests that the visual changes, like the basal metabolic rate, are functions of general metabolism. Similarly, it is known that the rate (although not the extent) of regeneration of visual purple *in vitro* is increased by raising the temperature of the system.[7] It may be stated, however, that maximal visual responses occurred in experiments 4 and 6 before changes in the basal metabolic rate took place. Moreover, no general correlation was found between the level of the basal metabolic rate and the speed or extent of dark adaptation in other subjects. In one patient with classical myxedema (B.M.R. –26) the dark adaptation was normal. In 4 control subjects, whose basal metabolic rates were –17, –19, –16, and –15, respectively, the dark adaptation curves were normal.

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[5] Fasold, H., and Heidemann, E. R., *Z. ges. Exp. Med.*, 1934, **92**, 53.

[6] Clausen, S. W., and McCoord, A. B., *J. Pediat.*, 1938, **13**, 635.

An increase in the circulating available Vitamin A does not account for the visual response, since in all experiments but one the serum Vitamin A fell to lower levels during therapy.

The improvement in speed and extent of rod adaptation was greater than that of cone adaptation. In this respect the response resembles that to Vitamin A therapy,[2] and is in contrast to the slight and equal lowering of the rod and cone thresholds observed to accompany recovery from anoxemia.[8] The changes appear, therefore, to represent an alteration in the speed and extent of visual purple regeneration rather than a local effect upon the nervous mechanism. The decrease in serum Vitamin A level accompanying the improvement in dark adaptation suggests that the feeding of thyroid extract or of α-dinitrophenol to these patients facilitated the utilization of Vitamin A by the visual mechanism.

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Hemolytic Effect of Gramicidin.

DOROTHY HEILMAN AND WALLACE E. HERRELL. (Introduced by J. L. Bollman.)

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Gramicidin, a bactericidal agent isolated by Dubos[1] from cultures of a sporulating soil bacillus, has a marked bactericidal effect against gram-positive bacteria, both *in vitro* and *in vivo*. However, this substance is very toxic for mice, rabbits and dogs when administered by the intravenous route. Early in the course of a study on the action of gramicidin by means of tissue culture methods, it was observed that gramicidin exerted a strong hemolytic action on rabbit's erythrocytes suspended in the tissue culture medium. The lots of gramicidin used in this work were furnished respectively by Dr. Rene J. Dubos and by Sharp and Dohme. The preparation of gramicidin used in this study was the alcohol-soluble, water-insoluble fraction which recently has been described and named tyrothricin by Hotchkiss and Dubos.[2] The tissue culture preparation employed in this study was similar to the one used by King, Henschel, and Green[3] to demonstrate hemolysin activity. The culture was planted on a 22 mm round coverslip and consisted of a drop of heparinized rabbit's plasma and 3 drops of tissue extract made by extracting 7-day chick embryos with rabbit's serum. A sufficient amount of rabbit's erythrocytes was added to make a 5% suspension in the final clot. Because gramicidin is insoluble in saline solution, suspensions were prepared by making suitable dilutions of a 2% solution of gramicidin in 95% alcohol with Tyrode's solution. Similar dilutions of 95% alcohol in Tyrode's solution were used as controls. In those cultures that contained 0.005 mg of gramicidin per cubic centimeter of culture medium, hemolysis was visible after incubation for 3 hours at 37.5°C, and the red cells were completely hemolyzed at the end of 16 hours of incubation. This amount, 0.005 mg of gramicidin, has been found to be bactericidal for pneumococci in this preparation.* Cultures containing 0.0025 mg of gramicidin showed complete hemolysis after incubation for 24 hours at 37.5°C. No hemolysis was observed in control cultures containing comparable dilutions of alcohol.

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[7] Zewi, M., *Act. Soc. Sc. Fenn.*, 1939, New Series B, **2**, 1.

[8] McFarlane, R. A., and Evans, J. N., *Am. J. Physiol.*, 1939, **127**, 37; McDonald, R., and Adler, F. H., *Arch. Ophth.*, 1939, **22**, 1.

[1] Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 1, 11; *Ann. Int. Med.*, 1940, **13**, 2025.

Greater concentrations of gramicidin were necessary to cause complete hemolysis of erythrocytes in blood agar plate preparations containing 0.6 cc of defibrinated rabbit's blood and 12 cc of nutrient agar. Preparations containing 0.05 mg of gramicidin per cubic centimeter of blood agar showed complete hemolysis only after 36 hours of incubation at 37.5°C. Small, circumscribed areas of hemolysis, apparently surrounding small aggregates of gramicidin were observed after 48 hours of incubation in blood agar plates containing 0.005 mg of gramicidin per cubic centimeter of medium.

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[2] Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1940, **136**, 803.

[3] King, J. T., Henschel, A. F., and Green, B. S., *J. A. M. A.*, 1939, **113**, 1704.

* To be reported.

Suspensions of 1% washed sheep's cells containing 0.01 mg of gramicidin per cubic centimeter were completely hemolyzed after one hour in a water bath at 37.5°C. Hemolysis was complete in 24 hours in tubes containing 0.001 mg of gramicidin per cubic centimeter of red cell suspension. The addition of small amounts of pooled guinea pig serum caused a slight but definite diminution of the hemolytic activity of gramicidin. The destruction of complementary activity of the serum by heating to 56°C for 30 minutes did not alter the inhibition of hemolysis. Attempts to neutralize this hemolytic effect by the addition of varying amounts of cholesterol to solutions of alcohol and suspensions of gramicidin were unsuccessful.

Further studies must be made to determine whether or not there is a relationship between the hemolytic effect of gramicidin and the hemolysin produced by the soil bacillus from which gramicidin is extracted. The description by MacLeod, Mirick and Curnen[4] of the effect of the intravenous administration of gramicidin on animals makes it seem likely that the hemolytic activity of gramicidin may play an important part in the toxicity of this substance. How far the hemolytic effect accounts for the total toxicity must be determined by *in vivo* experiments; however, the hemolytic activity of gramicidin is great enough in the presence of constituents of the blood to render inadvisable the intravenous use of this substance in bactericidal concentrations.

Summary. Gramicidin has a powerful hemolytic action against rabbit's and sheep's erythrocytes *in vitro*. This activity is marked even in the presence of serum, plasma and tissue extract. The presence or absence of complement did not appear to affect the process.

11931

In vitro Maintenance of Mammalian Endocrine Organs.

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The cultivation of endocrine organs was undertaken primarily with the hope that the secretory activity of such organs and their response to hormones might eventually be studied *in vitro*, under conditions probably less complicated than those occurring within the organism. In view of the contradictory nature of the results reported in the literature, it was first necessary to find a suitable reproducible method by which organs could be maintained structurally for varying periods of time. Recently, Parker[1] has described such a technic which has yielded good results for small fragments of such organs as liver, spleen, lymph node and thyroid. This method, which involves a fluid medium and the daily introduction of mixtures of oxygen and carbon dioxide, appeared best suited for our purpose. Results of the application of this method to the cultivation of thyroid, testis, ovary, certain reproductive accessories, anterior lobe of the pituitary and adrenal from immature and adult rats, guinea pigs and rabbits are given in Table I. Each experiment generally lasted 4 or 5 days. The flasks containing the tissues were kept at 38°. Microorganism contamination cases, which were seldom encountered in the entire investigation are not taken into account. All failures were due to unknown causes.

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[4] MacLeod, C. M., Mirick, G. S., and Curnen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 461.

The results given in Table I indicate that the methods described by Parker for the maintenance of adult tissues in a fluid medium may be successfully applied to certain of the endocrine organs. It also becomes apparent that different endocrine organs exhibit dif-

TABLE I.

Organ	Total No. exper.	No. successful*	Comments on successful experiments
Thyroid	20	16	Both follicular and interfollicular tissue in excellent condition.
Testis	15	11	Maintenance only; no advancement in spermatogenesis or development of interstitial tissue.
Ovary	15	7	Mature follicles, eggs and corpora lutea maintained 10 days.
Epididymis	8	7	Epithelium and connective tissue normal.
Fallopian tube	9	9	
Anterior hypophysis	20	17	Both chromophilic and chromophobic elements well maintained. Areas of growth occasionally noted.
Adrenal cortex and medulla	11	0	
Adrenal capsule	4	0	

*In each experiment, for all organs studied, the glands of a different animal were employed. By a successful experiment is meant one in which all or the greater number of cultured pieces were found in good condition.

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[1] Parker, R. C., *Methods of Tissue Culture*, N. Y., Hoeber, 1938.

ferent degrees of sensitivity to alterations in the environment. For example, we have found that thyroid structure can be maintained under a variety of conditions, *i. e.*, in plasma clots, in fluid media containing a small amount of serum or composed entirely of serum and at temperatures ranging from 25-38°C. The adrenal, on the other hand, cannot be maintained under *in vitro* conditions which are favorable for thyroid and pituitary, and apparently requires the presence of additional unknown materials. Mitotic figures and areas of proliferation were encountered only in the case of the anterior lobe of the pituitary. It would thus appear that the medium employed by Parker and in the present experiments is not only satisfactory for the maintenance of such organs as the thyroid, testis, and reproductive accessories, but also permits further growth of the anterior lobe of the pituitary.

In agreement with the results obtained by Parker, the use of autologous serum in our experiments was of no special advantage. In certain cases, sera obtained from other animals of the same species proved to be as satisfactory as serum from the same animal. In other experiments, degeneration occurred despite the use of autologous serum. It was also found that rabbit and guinea pig organs were more successfully cultured than those of rats and that, in most cases, young animal tissues thrived to a better extent than those from adults.

A puzzling observation was the frequent occurrence of many well maintained organ pieces lying near fragments showing almost complete necrosis despite the fact that all were of about the same size and continually bathed by the same medium. Perhaps different degrees of injury sustained by the organs in the cutting process may account for this finding.

The failure of adrenal capsule tissue to proliferate to any extent *in vitro* was surprising in view of the marked regeneratory capacity of this tissue within the organism. The rather inconsistent results obtained with the ovary are also difficult to understand. It is quite possible that the addition of pituitary organ pieces or extracts to the culture medium might enable such endocrine glands to be more successfully maintained. Our most immediate problems are thus concerned with determining the environmental conditions which are optimal for the culturing of the different endocrine organs.

Summary. 1. The methods of Parker which involve the use of a fluid medium and a high oxygen tension have been applied to the *in vitro* maintenance of endocrine organs. Best results were obtained with fragments of thyroid, testis, epididymis, Fallopian tube and the

anterior lobe of the pituitary of immature and adult guinea pigs and rabbits. 2. Inconsistent results were obtained with ovary. Adrenal tissue and capsule invariably underwent degeneration. 3. Evidences of growth (areas of proliferation and mitotic figures) were observed only in the case of the pituitary. 4. It is concluded that the conditions under which one endocrine organ may be successfully maintained *in vitro* are not necessarily the same as those required by another.

11932

Failure of Histaminase to Prevent Anaphylactic or Histamine Shock in Guinea Pigs.

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The increasing interest in the hypothesis that anaphylactic shock and allergy depend on the liberation of histamine has directed attention to the possibility of destroying this physiologically active substance *in vivo*. Best and McHenry[1] in discussing the enzyme, histaminase, suggest as its possible rôle the inactivation of histamine *in vivo*. In a more recent note, these authors[2] object to the misinterpretation of this suggestion and deny that available preparations of histaminase are, in their experience, effective *in vivo*.

Karady and Browne[3] were able to protect guinea pigs from the intraperitoneal injection of histamine by earlier intraperitoneal injection of histaminase. They were also able to protect sensitized guinea pigs from anaphylactic shock by treating the animals with histaminase prior to giving the shocking dose of antigen. These injections were given intraperitoneally.

The purpose of this report is to present experiments dealing with the *in vivo* action of a commercial preparation of histaminase* on anaphylactic and histamine shock in guinea pigs.

Methods. Guinea pigs averaging 350 g were sensitized by injecting 1.0 ml of 10% egg white intraperitoneally. At the time of giving the shocking dose of antigen, 3 weeks later, the guinea pigs were anesthetized by an intraperitoneal injection of nembutal. The trachea was exposed and a glass T-tube inserted. One outlet of this tube was partially closed by means of rubber tubing and a screw clamp, and the other connected to the tambour of a kymograph which recorded breathing continuously until bronchospasm occurred. This method is a modification of that of Auer and Lewis[4] used in studying anaphylaxis. A record of breathing during anaphylactic shock is an important aid in differentiating anaphylactic death from that due to emboli or hemorrhage. The sudden spasm of the bronchi with resulting arrest of breathing despite respiratory effort is a very striking and characteristic feature of anaphylactic or histamine shock in guinea pigs. Air does not pass in or out of the respiratory tract of shocked guinea pigs even when pressure is applied to the chest wall.

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[1] Best, C. H., and McHenry, E. W., *J. Physiol.*, 1930, **70**, 349.

[2] Best, C. H., and McHenry, E. W., *J. A. M. A.*, 1940, **115**, 235.

[3] Karady, S., and Browne, J. S. L., *J. Imm.*, 1939, **37**, 463.

* The histaminase used was a preparation of the Winthrop Chemical Company (Torantil-T360), obtained through the courtesy of the Department of Medical Research of that company.

Histaminase was injected intracardially into some of the animals 15 minutes or 24 hours before testing their reaction to the sensitizing antigen. The guinea pigs, their breathing having been recorded, were then injected with 0.5 ml of 10% egg white intracardially. Also the effect of the administration of histaminase on the subsequent injection of histamine hydrochloride dissolved in 0.85% NaCl

TABLE I.
Effect of Administration of Histaminase on Production of Anaphylactic Shock in Guinea Pigs.*

Units† histaminase administered	Time between administration of histaminase and shocking dose of egg white	Amt of 10% egg white sol. inj. intracardially as shocking dose, ml	Time in sec. before bronchospasm after inj. of egg white
5	15 min.	1.0	17
5	15 ”	0.5	27
8	15 ”	0.5	65‡
10	15 ”	0.5	25
12	15 ”	0.5	20
6	24 hr	0.5	23
6	24 ”	0.5	21
10	24 ”	0.5	22
10	24 ”	0.5	20
0		0.5	25
0		0.5	21

*The guinea pigs were sensitized 3 weeks previously by the administration of 1.0 ml of 10% egg white intraperitoneally.

†One unit of histaminase was defined by the manufacturer as the amount required to neutralize 1.0 mg histamine hydrochloride *in vitro*.

‡Questionable intracardial injection.

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[4] Auer, J., and Lewis, P. A., *J. Exp. Med.*, 1910, **12**, 151.

was tested in normal animals. Varying amounts of the histamine salt were injected intracardially. The smallest amount which consistently killed the animals was 0.2 mg. By making all injections directly into the blood stream, the uncertainty of varying absorption rate from the peritoneal cavity or subcutaneous tissue was eliminated. On the other hand, the rapidity of onset of shock, either anaphylactic or more particularly histamine shock, requires a rapid action of histaminase if it is to be effective.

Results. The data in Table I indicate that histaminase injected 15 minutes or 24 hours prior to giving the shocking dose of egg white did not prevent or alter the response of the sensitized animals. When normal guinea pigs were treated with varying amounts of histaminase at different time intervals before they were injected intracardially with histamine hydrochloride, it was found (Table II) that the histamine shock was unaltered by the previous treatment with large doses of the enzyme preparation. Fig. 1 illustrates the type of record of breathing obtained with guinea pigs during anaphylactic or histamine shock. During bronchospasm, indicated by the straight line portion of the record, the animals made sporadic respiratory efforts. During such efforts occasional, or no evidence of breathing is noted, depending on the severity of the shock. There was no observed alteration in the breathing pattern as a result of previous treatment with histaminase in sensitized animals injected with egg white or in normal animals injected with histamine. The *in vitro* activity of the histaminase preparation employed was verified after the completion of the experiments reported.

TABLE II.
Effect of Administration of Histaminase on Production of Histamine Shock in Guinea Pigs.

Units* of histaminase administered	Time between administration of histaminase and histamine	Amt of histamine inj. intracardially, mg	Time in sec. before bronchospasm after histamine inj.
5	2 min.	1.0	2
5	2 ″	0.1	2
2	12 ″	0.1	3
5	18 ″	0.2	3
5	22 ″	0.4	4
12	15 ″	0.4	4
6	1 hr	0.4	3
6	3 ″	0.4	3
6	24 ″	0.4	3
6	24 ″	0.4	3
0		0.4	3

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*See Table I.

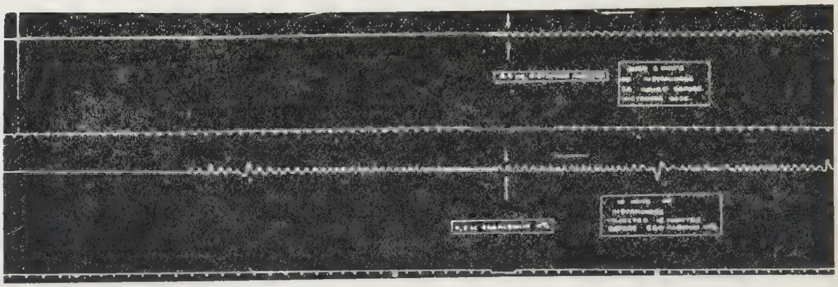

Fig. 1.

Conclusion. No evidence was obtained of *in vivo* activity of histaminase in preventing anaphylactic or histamine shock. There is still a possibility, however, that histaminase might act *in vivo* if the release of histamine into the body were slow and small in amount, thus allowing more time for the enzyme to act.

11933

Treatment of Experimental Hydrofluoric Acid Corrosion.

Aaron Paley* and Joseph Seifter. (Introduced by W. E. Hambourger.)

From the Department of Pharmacology, Western Reserve University, Cleveland, O.

In the experiments here presented hydrofluoric acid corrosions in rats were infiltrated hypodermically with a 3% solution of calcium gluconate in distilled water.

Adult rats were treated on the lateral aspect of the front paws just below the shoulder or on the lateral aspect of the thigh. The method was to clip but not shave the skin and to apply a drop of 40% hydrofluoric acid on the tip of a wax applicator to a spot of skin 7 mm in diameter. After a variable time interval, 0.5 cc of the calcium gluconate solution was injected subcutaneously under the lesion (or intramuscularly if the skin had sloughed off) in some animals, while in some the lesion was left untreated to serve as controls.

All the lesions, both treated and untreated, eventually healed, but there was a marked difference in healing time between the two groups. The criterion of healing was the complete disappearance of scab and granulation tissue. The average healing time of 21 untreated lesions is 17.6 days and the median 19 days. In the case of the treated animals a marked break in effectiveness of treatment apparently occurs between 8 and 16 hours. The average healing time of 19 lesions treated within 8 hours is 12.5 days; the average of 10 treated 16 hours or later after application of acid is 16.6 and the median 18 days, or essentially the same as for the controls.

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* Crile Scholar, Summer, 1939.

Another criterion of the usefulness of treatment is the incidence of suppuration. Pus was observed in 7 of the untreated animals (or 33.3%) at some time during their course, and these had an average healing time of 19.5 days. Only 3 of the lesions treated within 8 hours suppurated (or 15.8%). If the 7 suppurating burns be eliminated from the control series, the average of the remainder is 16.6 days (as compared to 12.5 for the treated). The effectiveness of treatment cannot then be wholly attributed to increased resistance to secondary infection. A series of 6 lesions were given from one to 5 additional injections at daily intervals. They showed no improvement over those receiving a single treatment, a result consistent with the finding that treatment after 16 hours is ineffective.

Discussion. With very sparing application of the acid a slight lesion was produced. There was a latent period of 10 minutes before the first objective evidence of tissue injury was observed, *viz.,* a gray discoloration at the site of application. Twenty minutes after application of the acid a slight wheal appeared, but at half an hour the skin looked perfectly normal, and not till one hour after the acid did a red spot of hyperemia develop. This became more intense and 48 hours later had an indurated feel. By the fourth day a definite red crust had formed which separated on the sixth day, revealing a practically healed base.

A more severe corrosion was produced with slightly more liberal application of acid. Within 5 minutes the gray zone was visible. At 10 minutes the gray area had a raised white areola about it. At 15 minutes the gray area was very sharply demarcated and the surrounding edema was more extensive and had a pink cast. At one hour the reaction (edema, erythema, and gray zone) appeared to have diminished, but deeper red spots soon appeared on the border of the gray zone. This lesion also progressed through intense hyperemia, induration, and crust formation. On the sixth day the crust came off revealing an ulcer into the subcutaneous tissues with a clean granulating base. A new crust subsequently formed and healing was not complete till the 15th day.

Greater uniformity of lesions could be obtained if they were made severe rather than slight or moderate. Where these appeared of unequal severity, the worse one was chosen for treatment and the other kept as a control, to keep possible errors on the side of conservatism. In severe corrosions the usual gray area appeared, but within one to two hours, sloughing occurred readily, so as to reveal the underlying muscle, covered only by a clear, more rarely a bloody serous exudate. Twenty-four hours later this had apparently condensed to form a hard crust and the skin margins were bound down and indurated. Healing proceeded by granulation under the crust, the final result being in every case a surprisingly insignificant scar.

Conclusions. 1. Infiltration of 3% calcium gluconate within 8 hours after intense application of hydrofluoric acid caused a significant shortening (about 30%) in healing time and a significant reduction (about 50%) in the incidence of suppuration. The latter cannot alone wholly account for the quicker healing. 2. Multiple treatment at 24-hour intervals is no more effective than a single treatment. 3. Rats appear to show a much quicker and less complicated recovery from severe hydrofluoric acid corrosion than humans. 4. The lesions heal characteristically by granulation under a hard crust.

We are indebted to Professor Torald Sollmann for his important suggestions.

11934 P

Rise in Serum Copper Following Oral Administration of Copper Sulfate.

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In a recent publication[1] we suggested that copper may be transported in the serum. The data presented in this paper support this conception. Using dogs as experimental subjects, we have been able to demonstrate a substantial increase of copper in the serum following oral administration of aqueous solutions of copper sulfate.

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[1] Sachs, A., Levine, V. E., Andersen, A. C., and Schmit, A., in press.

The dogs were kept in individual cages and were maintained at a constant weight on a Purina chow diet. They were fasted for 18 hours, following which the basal copper level in the serum was determined from hour to hour for 7 hours. The results indicate that fasting serum copper values remain practically constant. Several days later, at the end of another 18-hour fast, a sample of serum was collected. Then the copper sulfate ($CuSO_4 . 5H_2O$), dissolved in approximately 400 cc of distilled water, was administered immediately by stomach tube. Serum samples were collected one half hour later and at hourly intervals thereafter for 5 hours. The serum copper concentrations were determined by the method previously described[1] except that final color comparisons were made with a photoelectric colorimeter.

The effect of feeding varying amounts of copper sulfate is illustrated in Fig. 1. In each case the serum copper was raised definitely above the basal level. As the curves indicate, the larger doses of copper sulfate produced a more sustained rise in the serum copper than the smaller doses. The dogs retained 8 mg of $CuSO_4 . 5H_2O$ per kilo very well, but doses as large as 16 mg per kilo usually caused emesis. We shall determine the maximum dose which can be retained and which will at the same time produce the maximum rise in serum copper. We also plan to determine the site of absorption of copper from the gastrointestinal tract.

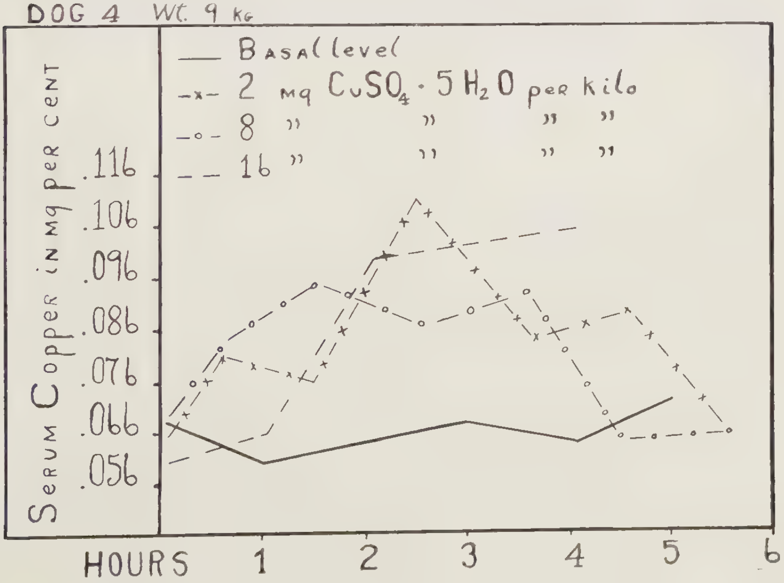

FIG. 1.

11935

Effect on Blood Picture of 15-Day Embryo of Liver Extract Injected into Pregnant Rat.*

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The morphological similarity of primitive, pre-hepatic erythropoiesis in the embryo and megaloblastic erythropoiesis in untreated pernicious anemia[1, 2] has prompted further study of the embryo with the view of developing a method of bioassay for the antianemia principle in liver extract. A relative deficiency of the antianemia principle may be the etiological agent in both types of erythropoiesis.[3] Extensive studies have been made on the response of newborn rats whose mothers have been treated with antianemia substances. Accelerated maturation has been reported[4-7] but has not been confirmed.[8, 9, 10] Recent observations of accelerated maturation of erythropoietic cells in the yolk sac of the 11-day rat fetus subsequent to liver and Ventriculin therapy in the pregnant rat emphasize the necessity of studying the prehepatic or "megaloblastic" generation of cells.[11, 12]

The circulating blood in the 15-day fetus is comprised predominantly of erythroblasts, normoblasts, and non-nucleated reticulocytes and erythrocytes of the prehepatic generation. Therefore, accelerated maturation of erythropoiesis in the 11-day fetus should appear in a study of the blood of the 15-day fetus. Since the per-

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* Aided by a grant from the Armour Research Fund of the University of Chicago.

[1] Kirschbaum, A., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 542.

[2] Schwarz, E., *Wien. Klin. Wchnschr.*, 1928, **41**, 192.

[3] Wintrobe, M. M., and Shumacker, H. B., *J. Clin. Invest.*, 1935, **14**, 837.

[4] Stasney, J., Higgins, G. M., and Mann, F. C., *Proc. Staff Meet. Mayo Clinic*, 1937, **12**, 699.

[5] Stasney, J., and Higgins, G. M., *Proc. Staff Meet. Mayo Clinic*, 1937, **12**, 490.

[6] Briese, E., and Higgins, G. M., *Anat. Rec.*, 1939, **73**, 105.

[7] Schlicke, C. P., *Am. J. Med. Sci.*, 1940, **200**, 155.

[8] Wigodsky, H. S., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 787.

[9] Bruner, H. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 260.

[10] Noble, T. A., Wigodsky, H. S., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 373.

[11] Jones, O. P., *Anat. Rec.*, 1939, **73**, 29.

[12] Jones, O. P., *Ibid.*, 1940, **76**, 39.

centage of non-nucleated cells rises rapidly between the 14th and 18th days of fetal life, a significant increase above the control percentage value at 15 days represents accelerated maturation of primitive erythropoiesis.

Methods and Technics. Young adult female rats were mated and on the 15th day of pregnancy the fetuses were removed under ether anesthesia with the amnion, yolk sac, and placental button intact. Fetal blood was obtained by puncturing the vitelline vessels. One sample of blood was smeared and stained with May-Grunwald-Giemsa stain. Supravitally stained wet preparations were also made by mixing another drop of blood with .18% brilliant cresyl blue in isotonic salt solution. 1000 cells per fetus were counted, 500 on the wet preparation and 500 on the dry smear. The choice of 4 fetuses per litter yielded a representative sample of the litter. The weight of each fetus was recorded, since the body weight was considered the most accurate gross measure of fetal development in the rat.[13] The rats were injected subcutaneously with 2 liver preparations at 4 dosage levels. The Lederle† liver extract contained 15 units per cc, whereas the Armour product‡ was assigned an arbitrary unitage upon the weight of fresh beef liver represented in each cc of extract. The animals were usually given 3.5 to 7.5 units on the sixth day of pregnancy and maintained on 1.5 u daily through the 14th day. Rats on the 31 u level received 7.5 u on days 6, 7, and 8, with subsequent 1.5 u maintenance dose daily. Thus the pregnant rats received liver therapy before, during and after the anlage of the mesoderm which gives rise to the blood-forming tissues in the fetus.

Results. Fig. 1 illustrates the percent non-nucleated cell values as plotted against fetal weight. Is the response of the animals receiving liver therapy biologically significant? The distribution of variation of the control animals contains 2 components, *i. e.,* the variation at any given fetal weight and the upward trend. Since the trend factor is fortuitous and unnecessary for comparison of a treated and untreated animal at any given fetal weight, it can be eliminated. By fitting a straight line to the control curve by the least squares method and by calculating the distribution of variation of control values from the straight line, the standard deviation can be determined independently of the trend. The means of percent counts on treated and untreated animals as compared with the

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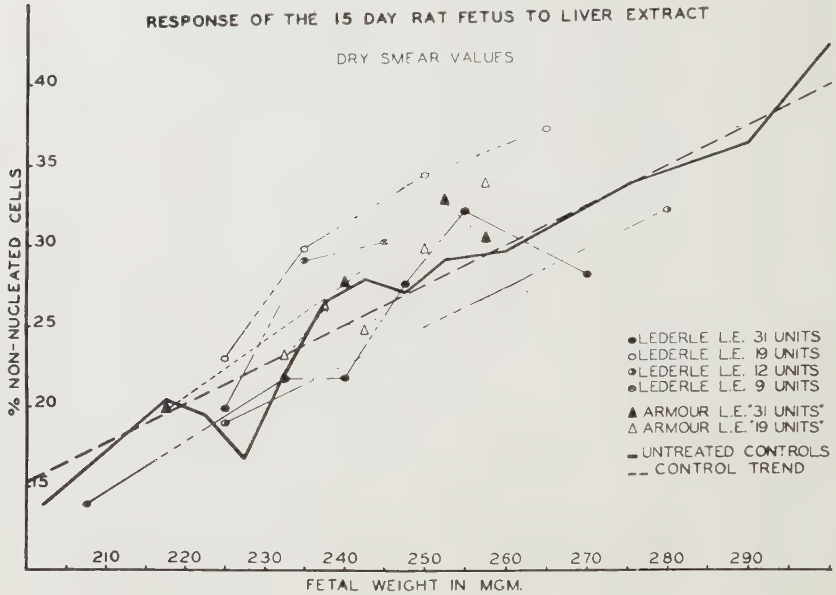
[13] Donaldson, H. H., *The Rat,* 1924.

† Generously contributed by Dr. Guy Clark of Lederle Laboratories.

‡ Obtained through the kindness of Armour Laboratories.



means of the calculated normal response determined from the straight line equation are listed in Table I. In no instance does a mean of a treated group of rats exceed the calculated control value by the conventional 2 sigma.

Summary. 1. Potent and standardized liver preparations were

TABLE I.
Comparison of Differential Counts on Treated and Control Fifteen-day Rat Embryos.

				% non-nucleated cells (mean values)			
				Wet smears		Dry smears	
Therapy	Litters	Fetuses	Mean fetal wt in mg	Expt.	Calc.*	Expt.	Calc.*
Controls (untreated)	30	120	240	22.4	22.4	26.2	26.3
				$\sigma = 3.3$		$\sigma = 4.4$	
Lederle Liver Extr.							
A. 9 units	3	12	240	19.6	22.2	18.7	25.8
B. 12 "	3	12	240	20.1	20.7	26.4	23.8
C. 19 "	6	24	240	25.9	22.1	30.0	25.4
D. 31 "	9	36	240	21.8	22.6	25.3	26.5
Armour Liver Extr.							
A. "19 units"†	8	32	240	26.4	22.6	26.5	25.4
B. 31 " †	7	28	250	29.9	24.6	32.0	28.6

*Means of values calculated from straight line equation fitted to control data by the least squares method where X = fetal weight and Y = % non-nucleated cells.

†A unit equivalent to fifteen g of fresh beef liver.

injected into normal pregnant rats before, during and after the anlage of the mesoderm in the developing embryo. 2. Differential counts were made on wet and dry smears of blood from 15-day fetuses and the percent of non-nucleated red cells present taken as an index of maturation of the blood picture. 3. In a series of 264 treated and untreated fetuses from 66 litters of rats, statistical analysis of the data indicates the response in liver-treated animals to be within the range of biological variation.

11936

Effect of Progesterone Anesthesia on Systemic Blood Pressure.*

Sydney M. Friedman. (Introduced by Hans Selye.)

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It has recently been shown by Selye[1] that steroid hormones may exert an anesthetic effect. Since it is known that anesthetics in general tend to depress the blood pressure, it appeared of interest to determine the blood pressure effect of progesterone anesthesia as compared to that of one of the standard anesthetics.

Eight female adult albino rats of an average body weight of 150 g (142 to 155 g) were given sufficient progesterone in an oversaturated solution (100 mg per cc) intraperitoneally to induce full surgical anesthesia, and the blood pressure was determined at 10-minute intervals throughout the anesthesia until recovery was complete. The same animals were similarly followed with nembutal anesthesia 24 hours later. Blood pressure recordings were made indirectly on the tail with an apparatus for non-anesthetized animals.[2] The total dose of progesterone ranged from 17 to 22 mg. Such a range was necessary since some of the animals are more resistant to this preparation than others. The total dose of nembutal (pentobarbital sodium) was 1/10 cc of a commercial preparation (Abbott) which contained one grain per cc.

Table I presents the data for systolic blood pressures obtained

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* The expenses of this investigation were defrayed by a grant received from the Schering Corporation of Bloomfield, N. J., through the courtesy of Doctors G. Stragnell and E. Schwenk who have also kindly supplied the progesterone used in these experiments.

[1] Selye, Hans, Proc. Soc. Exp. Biol. and Med., 1941, **46**, 116.

[2] Friedman, Sydney M., in press.

TABLE I.

Animal No.	1	2	3	4	5	6	7	8
Initial B.P.	106	106	106	106	106	106	106	106
Progesterone	95	90	125	*	80	*	90	94
Nembutal	89	95	92	80	*	92	*	106

*Blood pressure recordings were not obtained at this point.

initially as well as the average of readings taken during the course of full surgical anesthesia which occurred within 20 minutes of injection and lasted one to two hours. Blood pressure is expressed as mm of mercury.

In no case did progesterone depress the blood pressure to any greater degree than nembutal, and in one case anesthesia occurred despite a rise in pressure. With both agents a brief preliminary rise of 10 mm Hg was noted immediately following injection. In the case of progesterone, a sustained rise of 15 to 25 mm was frequently noted before anesthesia, especially in those animals in which the steroid was given in divided doses. Such pressor effects with progesterone were interesting since Grollman, *et al.*,[3] claimed to have obtained a rise in blood pressure almost to hypertensive levels with chronic injections of this steroid.

Summary. Progesterone in anesthetic doses given intraperitoneally does not lower the blood pressure to any greater degree than does nembutal. Anesthesia with progesterone occurs without relation to pressor or depressor effects elicited by this steroid.

11937 P

Tensile Strength of Tibiae of Healed Rachitic and Normal Rats.*

A. A. Schiller and H. C. Struck. (Introduced by C. I. Reed.)

From the Department of Physiology, College of Medicine, University of Illinois, Chicago.

The early observation of Clark and Mrgudich[1] that rachitic and healed rachitic rat tibiae had lost the preferred longitudinal orientation of the crystal micelles of the inorganic material prompted investigation of the tensile strength of healed rachitic bone as compared with normal controls. Albino rats from the stock colony were used. At weaning, 5 litters, comprising 30 rats were divided equally into control and experimental groups. The control animals were kept on the stock diet, while the experimental group was placed on the Steenbock-Black rachitogenic diet. After 41 days the experimental animals were severely rachitic, as shown by X-ray photographs. They were then given 250 units of calciferol by mouth and placed on the stock diet. Prompt healing of the rickets resulted. The animals were continued on the stock diet for 6 weeks after the calciferol was fed, and at the end of this time no roentgenological difference could be demonstrated between the experimental animals and their littermate controls. The rats were killed by a blow on the head and the tibiae immediately removed and dissected free from soft tissues for determination of tensile strength.

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[3] Grollman, Arthur E., Harrison, T. R., and Williams, J. R., Jr., *J. Pharm. and Exp. Ther.*, 1940, **69**, 149.

* Part of the expenses of this investigation were borne by a grant from the Nutrition Research Laboratories.

[1] Clark, G. L., and Mrgudich, J. N., *Am. J. Physiol.*, 1934, **108**, 74.

The apparatus used is a modification of a standard engineering machine for testing structural material.† The tibia is supported on 2 blunt wedges 10 mm apart, and a blunted knife edge is placed on the bone from above to apply pressure. By means of a motor and suitable reducing gear a heavy weight is slowly pulled across the apparatus so as to increase the pressure on the bone through the knife edge. A writing point records the excursion of the weight, so that the total load on the bone at any instant may be calculated. Simultaneously a record of the elasticity of the bone is obtained from a gauge graduated in 0.001 inch attached to the knife edge. From such records accurate load deflection diagrams of each bone may be made by triangulation.

Preliminary results obtained from the group of 30 rats thus far studied are summarized in Table I.

The difference in breaking weight between the healed rachitic and normal bones is, in the case of the male animals, 3.51 times the

TABLE I.
Breaking Weights and Deflections of Rat Tibiæ.

	No. of bones	Sex	Mean breaking wt, g	Std. dev.	Mean def. 0.001 in.	Breaking wt / Deflections*
Healed	10	M	9345	1142	15.1	619
Rachitic	20	F	8309	896	15.0	854
Normal	8	M	13359	977	13.6	982
	22	F	11527	869	10.7	1086

*This figure represents an approximate measure of the elasticity of the bones.

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† The machine was designed by Professor H. F. Moore of the College of Engineering of the University of Illinois.

standard deviation, and in the case of the females 3.59 times the standard deviation. Preliminary measurements of the cross section areas of a small number of the bones show that there is no significant difference between the healed rachitic and normal bones. While the array of results forms a somewhat skewed distribution curve, it is nevertheless probable that the differences in tensile strength between the roentgenologically healed bones and the controls are significant. Further studies are in progress.

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The Effect of Renin on the Cardiac Output.

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Since the original description of a pressor substance (renin) in the kidney by Tigerstedt and Bergmann there has been considerable speculation on the possible rôle that renin may play in the production and maintenance of experimental hypertension. As the pressor effect of renin can be obtained in perfused surviving organs and it has no effect on the isolated perfused heart,[1, 2, 3] it has been generally assumed that an increase in the cardiac output plays little or no part in the elevation of the blood pressure. Hessel[4] states that renin produces no increase in the cardiac output in dogs but gives no experimental data. The present study was carried out to determine what, if any, effect renin has on the cardiac output. Several determinations were made with tyramine hydrochloride, a drug which gives a pressor response similar to that of renin.

Method. The cardiac output was determined in one female and five male dogs by the use of the Fick principle following the method of Marshall.[5] All experiments were carried out in the morning under basal conditions. With the exception of three preliminary experiments the dogs received from 2.5 to 3.5 mg of morphine sulphate per kilogram intravenously, from 1½ to 2½ hours before the start of the experiment. The oxygen consumption was measured with a clinical spirometer, using a Blalock dog mask.[6] Blood pressure was taken by an auscultatory method previously described.[7] Blood samples of one cc were analyzed for total oxygen with the Van Slyke apparatus using the Van Slyke and McNeil technic.[8] A standard correction was made for the nitrogen content of the blood. Room and animal temperatures were taken at the beginning and end of the experiment. A typical experiment was carried out in the following manner. The oxygen consumption was first taken for eight minutes as a check on the state of relaxation of the animal. This was followed by a control cardiac output determination. Mixed venous blood samples from the heart were taken before the arterial samples and sometimes also afterward. Then 5 cc of renin (prepared after the method of Friedman and his collaborators[3]) was injected intravenously. Mixed venous samples were taken at various intervals after the establishment of a definite pressor effect. In a small number of experiments from 3½ to 5 mg of tyramine hydrochloride were injected in place of renin. One determination of the cardiac output was made after an injection of 3.8 cc of a purified renin.†

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* Work done during tenure of the Charles Klingenstein Fellowship.

[1] Tigerstedt, R., and Bergman, P. G., *Skand. Arch. Physiol.*, 1898, **8**, 223.

[2] Hill, W. H. Phillip, and Andrus, Cowles E., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 213.

[3] Friedman, B., Abramson, D. I., and Marx, W., *Am. J. Physiol.*, 1938, **124**, 285.

[4] Hessel, G., *Klin. Wohnschn.*, 1938, **17**, 843.

[5] Marshall, E. K., *Am. J. Physiol.*, 1926, **77**, 459.

Seventeen determinations of the cardiac output were carried out after 12 injections of renin and of these the greatest increase was 14% in 2 instances. Nine determinations of the cardiac output were carried out after 5 injections of tyramine. In 3 instances the increase was greater than 10%, namely 15%, 26%, and 31%. Figures 1 and 2 show that there is no significant relationship between the change in cardiac output, the time of venous sampling and the height of the pressor response. The pulse rate was almost always slowed at the peak of the pressor effect, later becoming more rapid than the resting rate. Respiration usually became more shallow at the peak of the pressor effect. This latter effect was noticed only after renin.

Discussion. A comparison of the basal cardiac output in each dog from day to day with those of Marshall[5] in unanesthetized dogs shows a greater degree of variation in this series. This is probably due to the fact that no attempt was made to train the animals, while the dose of morphine was so light that at times the dogs were somewhat excited. When ever the dog actually struggled the experiment was discontinued. As these were acute experiments it was felt that small daily variations in the basal cardiac output could have no effect on the results. An adequate check on the constancy of the control cardiac output was the fact that mixed venous samples taken on many occasions before and after the arterial sample invariably checked. Any change from the basal state while blood samples were being taken was indicated by reference to the first oxygen consumption period. There were minor variations in the cardiac output after the injection of renin, but they were not significant. Harrison and his coworkers[9] considered a deviation of ±10% to be within the limits of error of the method. The greatest increase (14%) was obtained after the injection of a large dose of a purified renin which gave a tremendous rise in the blood pressure (128). The only other increase of cardiac output above 10% (also 14%) was due solely to a change in the oxygen consumption. In all of the other instances there was no rise although the pressor response was adequate. A possible explanation of the drop in output found in one case (19.6%) may be that the renin used was a crude unstandardized suspension which contained many other substances.

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[6] Blalock, A., *J. Lab. and Clin. Med.*, 1927, **12**, 378.

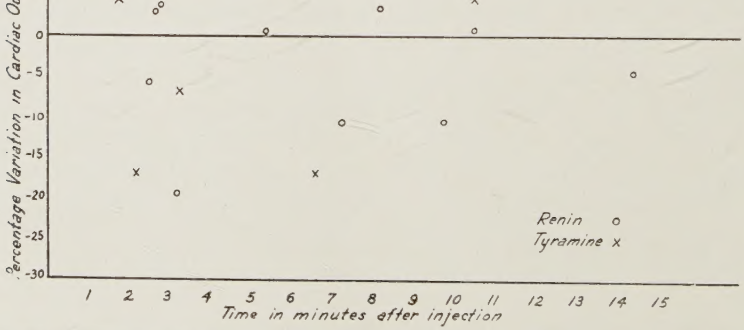
[7] Friedman, Ben, Somkin, E., and Oppenheimer, E. T., *Am. J. Physiol.*, 1940, **128**, 481.

[8] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, 1931, Vol. 2, p. 321.

† I am indebted to Dr. I. H. Page for this sample of renin.

Figure I

RELATIONSHIP BETWEEN THE % VARIATION IN CARDIAC OUTPUT AND TIME OF VENOUS SAMPLING



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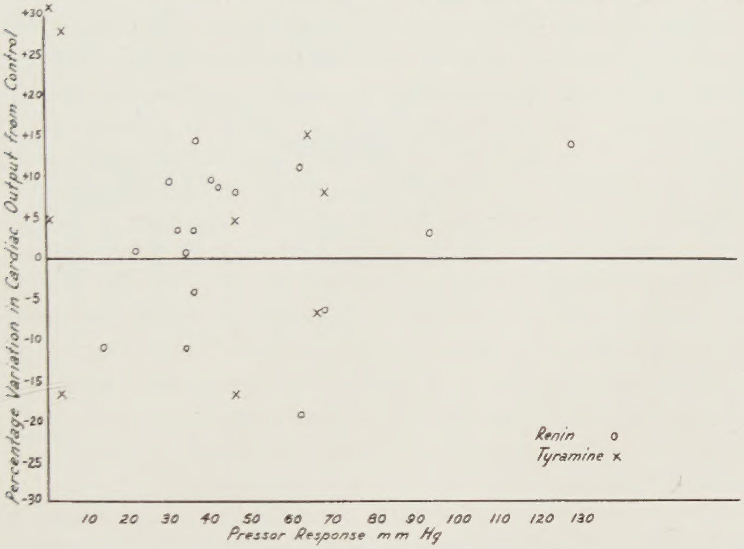
[9] Pilcher, Cobb, Wilson, Charles P., and Harrison, Tinsley R., *Am. Heart J.*, 1926-7, **2**, 618.

Figure II

RELATIONSHIP BETWEEN THE % VARIATION IN CARDIAC OUTPUT AND PRESSOR RESPONSE



There is an increasing amount of evidence to show that by injecting renin one can reproduce many of the physiological changes found in experimental and clinical hypertension. Hill and Pickering[10] found that a continuous infusion of renin gives a pressor effect which is well maintained for hours and that after stopping the flow, the blood pressure takes from 1 to 4 hours to return to normal. This is similar to the slow drop in blood pressure in the hypertensive animal after the removal of the ischemic kidney. Landis, Montgomery and Sparkman[11] showed that renin is the only pressor substance which does not lower the skin temperature. In the essential hypertension of human beings the skin temperature is normal. Smith and his collaborators have demonstrated that in essential hypertension and after the injection of renin it is the efferent arteriole of the glomerulus which is chiefly affected,[12] and Holman and Page[13] found that there was no change in cardiac output in dogs made hypertensive by the method of renal ischemia. Thus the fact that renin does not affect the cardiac output is an additional bit of evidence that this substance may be of significance in the production of experimental hypertension.

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[10] Hill, Janet R., and Pickering, G. W., *Clinical Science,* 1939, **4**, 201.

[11] Landis, E. M., Montgomery, H., and Sparkman, L. D., *J. Clin. Invest.,* 1938, **17**, 189.

[12] Smith, H. W., *Annual Review of Physiol.,* 1939, **1**, 503.

[13] Holman, D. V., and Page, I. H., *Am. Heart J.,* 1938, **16**, 321.

Previous reports on the effect of tyramine on the cardiac output are meager. Dale and Dixon[14] and Tainter[15] using the cardiometer reported an increased output but they give no data and base their opinion on the increased amplitude of cardiac pulsation which, however, cannot be used as an index of increased stroke volume, for this change may be demonstrated in a mechanical system with a fixed output, by increasing the peripheral resistance. Chen and Meek[16] in addition to the above method carried out two experiments with the teleroentgenogram. They report their results with this method to be inconclusive, as the variations fell within the limits of error. From the results obtained, using the Fick principle, tyramine would appear to have no direct effect on the cardiac output. The large increases which occurred in two instances were certainly secondary as they appeared only after the blood pressure had returned to normal.

Summary. 1. The cardiac output per minute of intact dogs anesthetized with 2.5 to 3.5 mg of morphine sulphate per kilogram was determined according to the Fick principle before and after the intravenous injection of renin and of tyramine. 2. On using a dosage of renin and of tyramine sufficient to give a good pressor effect, the cardiac output was not found to be significantly changed.

I wish to thank Drs. B. S. Oppenheimer and B. Friedman for their interest and criticism of this work.

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[14] Dale, H. H., and Dixon, W. E., *J. Physiol.*, 1908, **39**, 25.

[15] Tainter, M. L., *J. Pharm. and Exp. Therap.*, 1927, **30**, 163.

[16] Chen, K. K., and Meek, W. J., *J. Pharm. and Exp. Therap.*, 1926, **28**, 59.